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HEREDITARY MULTIPLE EXOSTOSIS

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Multiple exostosis in its severer form is a rather conspicuous skeletal disorder, whose characteristic knobby protrusions, especially those in the metaphysial regions of the long bones, must have intrigued even the earliest medical observers. It still evokes considerable interest, as is evident from the steady accumulation of titles relating to it in the *Quarterly Cumulative Index Medicus* and from the fact that clinical accounts of even one or just a few cases are held to be worth recording. Much is known about the disorder, and one of its most arresting and well worked-out features is the strongly hereditary character. The weakest link in the chain of knowledge about the condition is that of pathogenesis. This weakness stems from the lack of satisfactory anatomic studies of entire affected bones from the crucial peroid of infancy and very early childhood, which alone could yield conclusive findings on this aspect.

NOMENCLATURE

The earlier modern references to the disease favor the name "multiple cartilaginous exostoses" (or "exostosis") [1] in accordance with the precedent set by Astley-Cooper, or simply "multiple exostoses" [2] in agreement with Virchow. Even before the turn of the century, however, recognition of heredity as a factor in the disorder came frequently also to be expressed in the name. After the turn of the century many papers appeared describing the condition under the names of "diaphysial aclasis" and "hereditary deforming chondrodysplasia." The former designation was popularized by Keith [3] and still is commonly used in British journals. The latter designation was popularized by Ehrenfried [4] and is commonly used in the journals of this country. In addition to the names mentioned, a number representing merely variations of them have also been used.

The name "hereditary multiple exostosis" is being given preference in the present article because it seems to create a reasonably vivid picture of the disease as a whole without being too long. The term "diaphysial aclasis," while valuable in pointing out that the modeling of the entire area of affected bone is abnormal, emphasizes pathogenesis to the exclusion of concrete appearances. "Hereditary deforming chondrodysplasia" is vague and again creates no mental picture of the disorder in general or of its individual lesions.

Finally, there are two other names which require brief comment. One frequently encounters now descriptions of the condition under the heading "Dyschondroplasia." To apply the latter term in this connection is incorrect, as it

From the Laboratory Division of the Hospital for Joint Diseases.

1. (*a*) Henking, F.: Virchows Arch. f. path. Anat. **77**:364, 1879. (*b*) Huber, K.: ibid. **88**:256, 1882.

2. (*a*) von Recklinghausen, F.: Virchows Arch. f. path. Anat. **35**:203, 1866. (*b*) Cohnheim, J.: ibid. **38**:561, 1867. (*c*) Chiari, H.: Prag. med. Wchnschr. **17**:403, 1892.

3. Keith, A.: J. Anat. **54**:101, 1920.

4. Ehrenfried, A.: J. A. M. A. **68**:502, 1917.

was coined by Ollier in relation to cases of predominantly unilateral skeletal enchondromatosis. The fact is that within a short space of time Ollier wrote two articles—one on "multiple osteogenic exostoses" [5a] (the condition in question here) and one on "dyschondroplasia" [5b] (multiple enchondromatosis). Although Ollier himself kept the two disorders perfectly distinct, certain confusions have arisen through failure to read his articles carefully. They have resulted not only in the misapplication of the term "dyschondroplasia" to the disorder under discussion but also in an unfortunate concept expressed in the term "osteogenic disease," which is currently commonly used in the French and Italian literature. As used the term "osteogenic disease" is intended to imply the existence of a general disorder which may express itself in the appearance of either multiple exostosis or multiple enchondroma and is also believed to be commonly manifested in the appearance of both disorders in the same person. Actually there is no anatomic basis for the idea that in cases presenting multiple exostosis the bones often also show multiple enchondroma, and vice versa (page 354).

CLINICAL ASPECTS

Incidence.—The present discussion of multiple exostosis is based largely on the study of 28 patients actually treated at the Hospital for Joint Diseases and does not include any with the disease among the patients' relatives who were not themselves treated here. Though not exactly common, the disorder is by no means rare. Indeed, it is the most common of the systematized anomalies of skeletal development encountered clinically. This is understandable in the light of the absence, in connection with this disease, of an inherent lethal factor such as is present, for instance, in achondroplasia, osteogenesis imperfecta and osteopetrosis. Specifically, the disorder constitutes no special hindrance to maturation or even birth of the stigmatized fetus; the postnatal skeletal abnormalities characterizing it offer only exceptionally (at least until adult life is reached) any threat to continued existence (notably through evolution of one or more of the lesions into chondrosarcoma); and, even later on, relatively few subjects are destroyed by the disease.

Males predominate among the affected subjects. This is likely to be much more evident if the sex incidence is calculated on the basis of cases in groups of family trees than if one considers merely collected individual cases or groups of individual cases. As calculated on the former basis the ratio of males to females affected is about 7:3.

Emergence and External Aspect of the Disorder.—Apparently it is rare that the stigmatized infant presents any outward manifestations of the disease at birth. Whether in a family in which the disorder is known to be present one could pick out regularly by roentgenography those newborn infants in whom the disorder will subsequently become grossly manifest, one cannot say on the basis of the data available. In most cases the disorder comes to light during childhood, but there can be no doubt that in a good many (especially if it occurs in a mild form) it is never identified clinically at all.

In the cases clinically recognized, one or more "bumps" on the skeleton have usually been discovered accidentally by the patient or a relative. The data collected in the Hospital for Joint Diseases are in harmony with the idea that it is only exceptionally that the disease comes to light in this way before the patient is 2 years of age. However, from then on until puberty, but more particularly during the earlier years of childhood, the condition in the large bulk of the clinical cases is thus

5. (*a*) Ollier, L.: Lyon méd. **88**:484, 1898; (*b*) Bull. de chir. de Lyon **3**:22, 1900.

brought to medical attention. The discovery of a "bump" on some bone is followed in the course of time by the appearance and recognition of similar lesions on other bones. As the records in this hospital also show, there may be an interval of months or years between the time when lesions are first noted and the time when medical advice is first sought in regard to them. In a general way this lag is a consequence of the relative innocuousness of the condition in young children, and in addition it may result from the fact that a similar condition is present and has proved harmless in one or more adult members of the family. After adolescence growth of all the exostoses slows down, and during adult life no new ones form.

If the case is not brought to light before puberty, it is not likely to come to medical notice at all (except accidentally) unless or until in adult life attention is sought for the correction of some secondary deformity (for instance, shortening and curvature of the forearm) or on account of the appearance of some serious complication (such as the development of chondrosarcoma in a previously quiescent exostotic bone area).

The histories in the cases on which this article is based revealed that the presenting protuberance is not infrequently on a scapula (particularly along its vertebral margin) but is more commonly to be felt on the metaphysis of some long bone, especially in the region of the knee, the ankle or the shoulder. As more and more exostoses appear, the tendency of the condition to involve the long bones heavily becomes increasingly apparent. In the course of childhood a subject may come to have a considerable number of obvious prominences on the bones, presenting, indeed, a knobby appearance (fig. 1*A*). In the cases in which the involvement is severest, exostoses will be present also on the juxtaepiphysial regions of the metacarpal and metatarsal bones and even on the phalanges (fig. 2*A*), as well as on ribs, innominate bones and vertebrae. Even in these cases they are rarely found on skull bones. In general, the exostoses tend toward a bilateral and symmetric distribution, but in an occasional case one side of the body is found clearly more severely affected than the other.

In addition to presenting a knobby appearance, the more severely affected subjects may show definite shortness of the limbs in relation to the trunk. Consequently, the superficial appearance may resemble that of mild achondroplasia, but the patients do not present other stigmas of that disease. The shortness of the long bones is due in the main not to direct interference with longitudinal growth at the epiphysial cartilage plates (as in achondroplasia) but to dissipation of the longitudinal growth force in a lateral direction at the metaphyses. Through the supplementary agency of curvature and deformity of the forearm, one or both upper limbs are likely to be even more strikingly shortened than the lower.

Clinical Difficulties.—In the early stages of the evolution of the disease there are rarely any subjective complaints—even of discomfort or inconvenience—relating to it. However, sooner or later most of the subjects experience at least some minor difficulty. For instance, a large exostosis may cause discomfort from direct pressure on the neighboring tissues, though this is often mitigated by the development of a bursa between the growth and the overlying soft parts. Prominent exostoses around the ankle, the knee or the hip may not only cause pressure pain but may hinder full normal articular function or gradually disturb the stance and gait. Some subjects present curvature of the tibia and fibula. Disproportionate shortening of the lower limbs may result in some degree of scoliosis.

Deformity of the forearm, which Hagen [6] was one of the first to discuss in detail, is a disturbing complication, to be noted in at least one third of any repre-

6. Hagen, F. B.: Arch. f. klin. Chir. **41**:749, 1891.

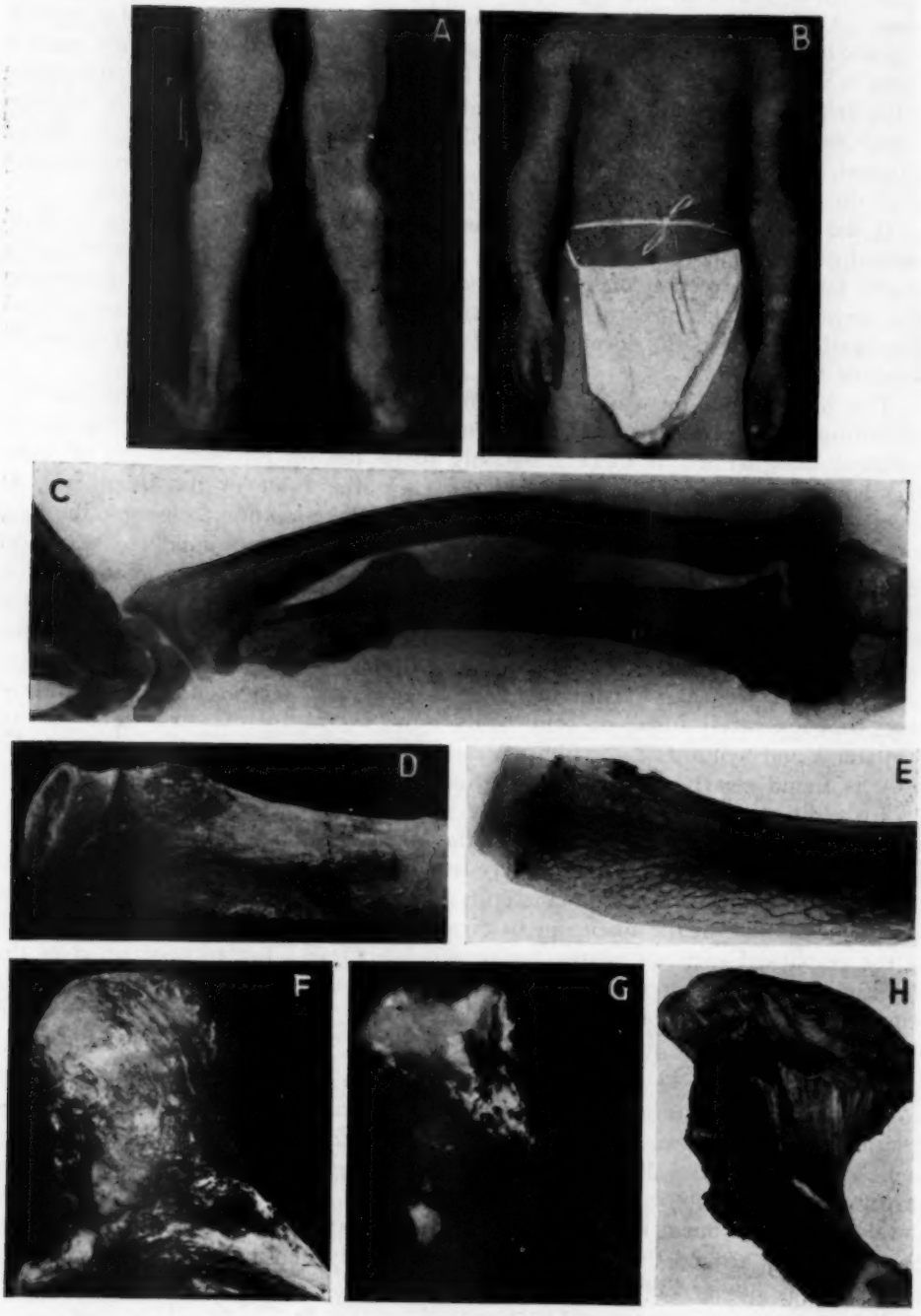

Figure 1

(See legend on opposite page)

sentative group of cases. Characteristically in connection with this deformity (figs. 1 *B* and *C*) one makes the following observations: The ulna is strikingly shortened, and its exostotically deformed distal end is more or less thimble shaped or conical; the radius, much less shortened than the ulna, is bowed outward, and the head of the radius may be found more or less dislocated outward and posteriorly. The factors operating in the causation of this deformity are striking retardation of growth at the lower end of the exostotically affected ulna (where about three fourths of the longitudinal growth of this bone normally occurs) associated with a much lesser degree of retardation of growth of the corresponding end of the less severely affected radius (where only about one fifth of the total longitudinal growth of this bone takes place). The outward bowing of the radius represents an attempt at adaptation to the shortening of the ulna, and when the proximal end of the radius has become completely dislocated the bowing of this

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EXPLANATION OF FIGURE 1

A, photograph of the lower limbs of a boy of 15, showing the bulges and general knobby appearance characteristic of multiple exostosis. Protuberances on the bones had been known to be present at least since the age of 10, but none had caused difficulty until the past few months, when the boy began to complain of pain on the inner aspect of the right ankle. The boy was of short stature for his age. His parents and his sister seem to be clinically free of exostoses, but nothing is known of the status of other relatives.

B, photograph illustrating the appearance of the upper limbs of another boy of 15 with multiple exostoses. Both of these limbs are definitely short in relation to the trunk. In addition, the right is shorter than the left and also more obviously deformed. The boy's brother and father showed the disease, and an uncle, a grandfather and a granduncle (all on the paternal side) were reported as having it.

C, roentgenogram showing the characteristic deformities of the forearm bones. Note the strikingly shortened ulna and the generally altered shape of its exostotically deformed distal third. Note also that the radius is disproportionately long in relation to the ulna, that it is bowed and that its head is dislocated. Pronation and supination of the forearm were considerably restricted, and motion of the elbow was also somewhat restricted. The patient was a woman of 26, who stated that she had been aware of an abnormality of the forearm since early childhood.

D, photograph of part of one of the many exostotic ribs removed at autopsy in the case of a man with multiple exostosis. Note that not far from the costochondral junction, at the upper margin of the picture, the rib shows a small protuberance, the crown of which was capped with cartilage. Farther along the body of the rib, two additional small and wide-based protuberances are plain, and these too had small shells of cartilage on their crowns. The chains or wreathlets of tiny knobby protuberances which were obvious near the costochondral junction on some of the ribs are not conspicuous on this one. The patient died of chondrosarcoma, which started in the left sixth rib.

E, roentgenogram of the rib shown in *D*. Though the exostoses are clearly represented in this postmortem specimen, it is unlikely that in clinical roentgenography exostoses of this size would be clearly visualized on the film. Nevertheless, even such small exostoses may be the point of departure for chondrosarcoma such as developed in the sixth rib in this case.

F, photograph (natural size) of a wide-based protuberance springing from the left seventh rib at the costochondral junction. This rib lesion was removed because it protruded into the breast and had been painful for the past three years. The patient was a woman of 22 in whom skeletal outgrowths had already been noted at the age of 2.

G, photograph (about natural size) showing the cut surface of the specimen illustrated in *F* after it had been sectioned in the transverse plane. The outgrowth itself has a thick cap of cartilage which at the right shows an area of cystic softening. The dull whitish area below and to the right of this represents a focus of calcified cartilage and calcareously impregnated cartilage matrix. The small semilunar white area in the lower left hand portion of the picture represents costal cartilage, showing the relation of the outgrowth to the costochondral junction.

H, photograph (natural size) of an osteocartilaginous exostosis removed from the outer surface of the body of a scapula. At the lower right hand corner of the picture a bit of the epiphysis for the vertebral border can be seen. The patient was a boy 9 years of age, and this growth was the presenting protuberance, first noted several years previously.

bone is likely to be found less pronounced. When the shortened and deformed lower end of the ulna no longer articulates properly with the bones of the wrist, the hand comes to deviate toward the ulnar side. In consequence of the exostotic changes immediately above the wrist in the radius and the ulna, blocking interference with supination and pronation of the forearm may develop. There are occasional variations of this typical pattern of deformity of the forearm, referable mainly to relatively greater severity of involvement of the radius as compared with that of the ulna.

The characteristic forearm deformity was present at least in mild form in 11 of the 28 cases. Although the severity of involvement was at times approximately equal in the two forearms, it was usually not so, and sometimes one forearm did not appear clinically to be deformed at all. A deformity of one or both of the forearms may already begin to be noted fairly early in childhood. It is to be found in practically all cases presenting pronounced skeletal involvement by multiple exostoses. Furthermore, exostotic involvement of the forearm and consequent deformity of it is seen even in some cases in which the rest of the skeleton is relatively lightly affected.

The complication of the disorder by cancerous change of one or more of the exostoses is given separate discussion later (page 347). It is likely that in most of the cases in which an outgrowth has been reported as causing difficulty by impinging on or compressing a peripheral nerve, a large blood vessel or a part of the spinal cord, this complicating difficulty is due to enlargement of the exostotic area in question by cancerous transformation.

PATHOLOGY

Topographic Anatomy.—If a patient with full-fledged classic multiple exostosis comes to autopsy, one cannot fail to note how much more extensive is the skeletal involvement than it appeared to be on the basis of clinical observation or even roentgenographic examination. Under these circumstances, in addition to numerous obvious exostotic outgrowths on the bones there are great numbers of small and even tiny excrescences, difficult to evaluate and count. Indeed, it is probable that no two observers would arrive at the same total figure in any particular case, since there would frequently be differences of opinion as to what to count as an individual lesion.

Whatever their numbers in any instance, the exostoses and excrescences tend to follow a definite pattern of distribution on the individual skeletal parts affected. Specifically, it is apparent that they develop particularly (though not solely, as I shall point out) in relation to sites of bone growth at epiphysial cartilage plates, appearing mainly as outgrowths on the bodies of the bones, in the general vicinity of the plates. Furthermore, there is a general positive relation between the severity of involvement of a particular juxtaepiphysial part and the activity of growth in that part. Thus it is not surprising that it is the long tubular bones and especially those of the lower limbs that show the most exuberant involvement. Again, it is natural that, on the other hand, the bones of the trunk should show less florid involvement than the long bones and that the vertebrae in particular should be even less severely affected than the other trunk bones. Though, as noted, the distribution of the lesions tends to be bilateral and symmetric, there are highly exceptional cases in which one side of the body is more severely affected than the other. This was true in the case described by Huber,[1b] for instance, in which, furthermore, the ribs and the short tubular bones were more heavily involved than the long tubular bones.

There are relatively few complete accounts of the anatomic findings relating to the skeleton in cases of well developed multiple exostosis. In this connection one can still turn with profit to the old descriptions by Recklinghausen [2a] (in the case of a man of 28), Cohnheim [2b] (in the case of a man of 22) and Chiari [2c] (in the case of a girl of 19). These authors gave abundant details, especially in regard to the topography of the lesions, and their cases are particularly illuminating from this point of view. However, in subjects of these ages the bones no longer show floridly evolving exostoses, so that in regard to the genesis of the lesions they yield but little information. This statement applies also to the 2 patients in the present series who came to autopsy, since they were respectively 32 and 49 years of age at the time of death.

Even in a case in which the skeletal lesions are widespread and well developed, the skull bones, as already indicated, are likely to be found exempt. However, the base of the skull, which is preformed in cartilage, has sometimes been found affected.[7] Specifically, in an occasional case one or more tiny or even pea-sized excrescences, usually composed entirely of cartilage (ecchondrosis), have been noted, particularly on the clivus or along the synchondrosis between the sphenoid and occipital bones. In regard to parts of the skull preformed in membrane there is no unequivocal evidence of exostosis formation. The carpal and tarsal bones on the whole are also spared; the exception is the os calcis. (The latter, since it develops a secondary center of ossification posteriorly, may show a few small exostoses on the main body of the bone.)

On the other hand, the bodies of the ribs are likely to show little wreaths of small pointed or warty cartilage-covered exostoses, especially at the chondral end but also, though to a lesser extent, posteriorly, particularly where the head of the rib articulates with the neck. Also, there are usually some small, flat, broad-based exostoses further along the rib body both anteriorly and posteriorly (figs. 1 *D* and *E*). Furthermore, the bodies of certain ribs may show in addition one or two relatively large sessile or stalked exostoses (sometimes situated even as far out as the midaxillary region) projecting either externally or into the pleural cavity (figs. 1 *F* and *G*). Not far from the costochondral junctions the costal cartilages themselves may show a number of pinhead-sized or even much larger bits of cartilage under the perichondrium (ecchondrosis), but the sternal ends are generally free from them. It is not at all likely that the sternum itself or the clavicles will show many exostoses. If any are shown by a clavicle, they will be at the sternal end of the body and will be few and small.

The scapulas are likely to be definitely affected. There may be a more or less continuous row of flat knobby exostoses protruding from the body both anteriorly and posteriorly along the line of attachment of the epiphyses for the vertebral border and the inferior angle. Not infrequently, also along this line on the body there are one or more exostoses so large and prominent that they have often been the ones which first directed clinical attention to the condition (fig. 1 *H*). Still on the body, along the line of attachment of the epiphyses for the acromion and the coracoid process one may also note some small warty exostoses. Like the scapulas, the innominate bones generally show rows of flat or knobby cartilage-covered excrescences located peripherally along the attachments of the secondary centers of ossification (the epiphyses) to the main bodies

7. Müller, E.: Beitr. z. path. Anat. u. z. allg. Path. **57**:232, 1913-1914.

of the constituent bones. In regard to these bones it will be found that on the whole the exostoses are more prominent and numerous on the anterior and posterior surfaces of the ilium, along the line of attachment of the epiphysis for the crest.

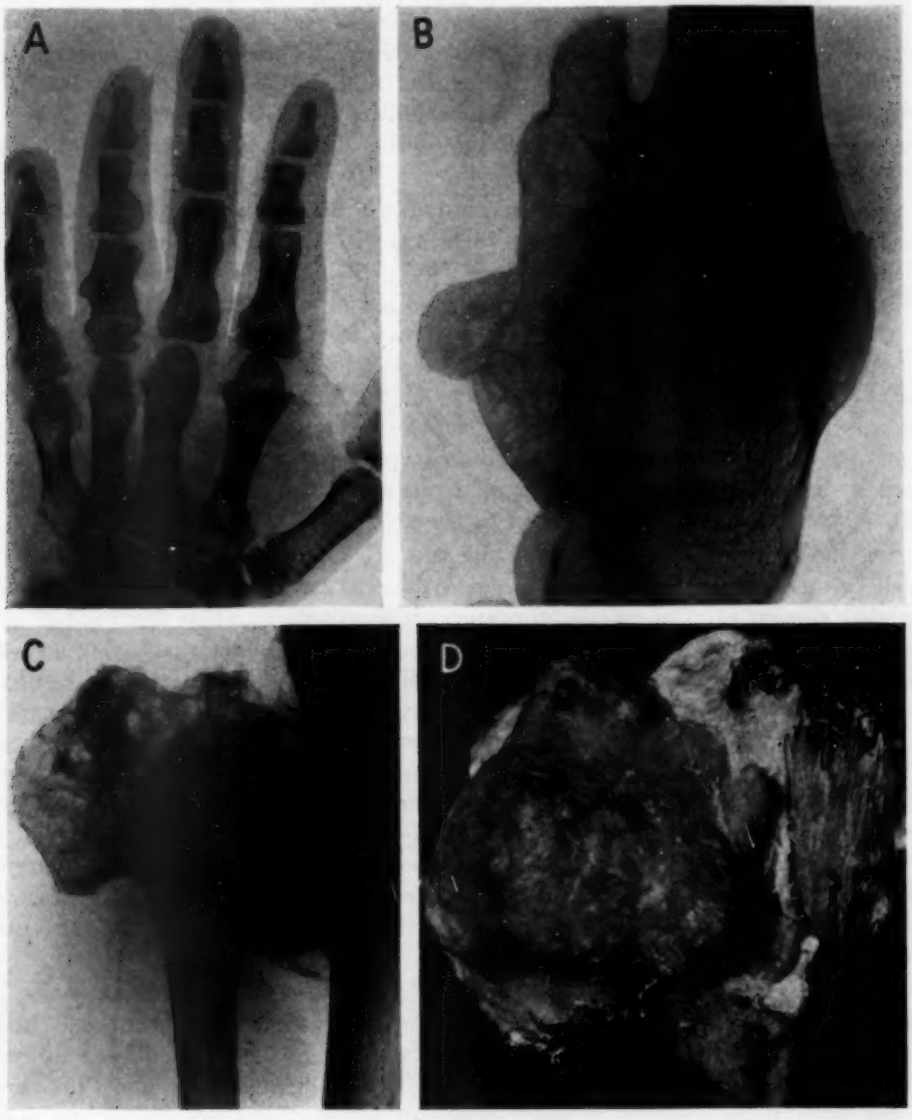

Figure 2

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Such few exostoses as are present on the vertebral column obey the general rule in being located close to the secondary centers of ossification (epiphyses). These exostoses are small, on the whole, and are located most commonly near the tips of spinous processes.

The phalanges and the metacarpal and metatarsal bones may also show some exostotic outgrowths on their shafts (figs. 2 *A* and 5 *A* and *B*).

As already noted, the fullest expression of the disorder is encountered in the long bones. In the lower limbs (in contrast to the upper) both the lower and the upper ends of the long bones will usually be found rather severely affected, although the involvement is likely to be heaviest in the regions of the knees. Because of the impingement of large exostotic outgrowths on each other at both the upper and the lower ends of the shafts of the tibia and the fibula, these bones may be found interlocked or even synostotic over several inches at these ends. In the humeri, though the upper juxtaepiphysial ends of the shafts are likely to be considerably altered, the lower ends are usually but slightly affected if at all. On the other hand, the upper ends of the shafts of the radiuses and the ulnas are likely to be found less affected than the lower ends.

Gross Pathology as Exemplified in an Affected Long Bone.—If a rather strikingly affected long bone is examined (figs. 2 *B*, *C* and *D*), it will be found that over a considerable distance away from each epiphysial end the shaft is wider and blunter than it would normally be. In fact, instead of tapering toward the middle of the diaphysis, the juxtaepiphysial regions of the shaft appear more or less cylindric or even ballooned out. Furthermore, instead of being smooth, the surface is knobby and bumpy over at least a large part of the circumference of these regions. This abnormality of the bone's outline may be accentuated by the presence of one or more pedicled knobby protuberances or of tubular projections ending bluntly or in a hook or a point. It is worth noting that on the whole the nodosities and projections tend to curve away from, rather than to envelop, the epiphysial ends of the bone. It is true that the modified juxtaepiphysial region may so expand as partly to encuff and even deform the epiphysis, especially in the upper end of the fibula and the lower end of the ulna. Nevertheless, even then the epiphysial end remains distinct from the encuffing metaphysial outgrowths (fig. 4 *D* and *E*).

Areas in a long bone that have been modified by the presence of large exostoses, especially if the latter have displaced and pressed on overlying muscle masses, may be found covered by bursas. Specifically, a more or less vascularized con-

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EXPLANATION OF FIGURE 2

A, roentgenogram of a hand in the case illustrated in figure 1 *B*. It shows exostoses on the shafts of the phalanges and metacarpal bones. The corresponding bones of the foot in this case showed similar outgrowths (fig. 5 *A* and *B*).

B, roentgenogram of the lower end of the right femur in the case illustrated in figure 1 *A*. Note the lobulated protrusions from the posterior surface of the metaphysis. The metaphysial region which appears to be outlined by the radiopaque line must represent another area of exostotic protrusion, but a different view would be necessary to show exactly where on the metaphysis it is located. Such extensive modification of a whole metaphysis is not unusual in advanced multiple exostosis.

C, roentgenogram showing the strikingly modified upper end of the right fibula in the case illustrated in figure 1 *A*. Exostoses from the abutting portion of the tibia, interlocking with those from the fibula, have resulted in a synostosis, so that when the affected upper end of the fibula was resected (fig. 2 *D*) a part of the adjacent portion of the tibia had to be removed also. As examination of the resected specimen showed, the radiopaque patch in the left upper corner of the picture represents a focus of highly calcareous cartilage and cartilage matrix (fig. 3 *B*). Though it penetrates deeply into the interior of the affected area, the calcareous focus is still continuous with cartilage capping the exostotic area in that region.

D, photograph (somewhat reduced) of the resected specimen in the case illustrated in *C*. The view is such that the small portion of tibia adherent to the distorted upper end of the fibula does not show. It can be seen that the distention of the fibula is not uniform, though even that portion in which the circumference is not ballooned out shows small protrusions.

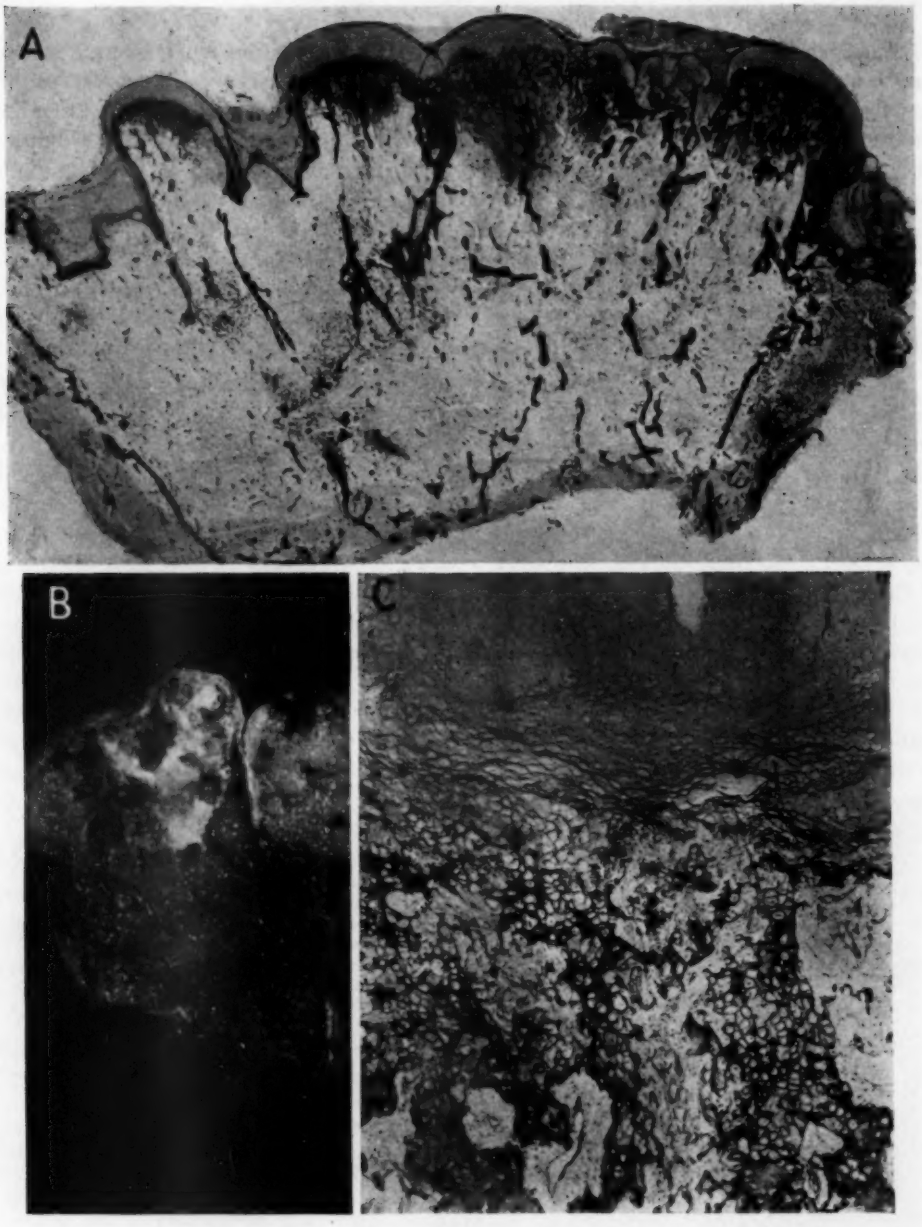

Fig. 3.—*A*, photomicrograph (× 2.5) showing a part of the exostotically bulged out juxta-epiphysial portion of the right femur in the case illustrated in figure 1 *B*. The ostectomy consisted of removal of a mass of bone approximately 6 inches (15 cm.) long, 3 inches (7.5 cm.) wide and 2 inches (5 cm.) high from the posterior aspect of the femur. The outer fibrous covering had been stripped away from the exostotic mass. Much of the surface of the outgrowth is still capped by cartilage, and the affected area had still been growing for the most part by the process of endochondral ossification.

B, photograph (about natural size) showing a gritty focus such as is sometimes found within an exostotic area. The illustration represents a transversely cut surface from the specimen illustrated in figure 2 *C* and *D*. The relation of this focus (appearing as a white patch) to the surface of the lesion is clear.

C, photomicrograph (× 20) from a smaller calcified focus in a lesion in another case. Note the hyaline cartilage above (peripheral cartilage cap) and the calcified cartilage and cartilage matrix below but continuous with it.

nective tissue membrane a millimeter or more in thickness may come to be formed between the modified area of the bone and the overlying muscle. The membrane is attached all around the base of the modified area, forming a sort of encasing bag, and in the latter fluid (sometimes containing fibrin) may accumulate so that the muscle is more or less cushioned from the outgrowth. Occasionally the lining in such an "exostosis bursata" becomes villously modified, and the sack may even come to contain a number of calcified bodies such as one sees in certain arthritic joints.

If one starts stripping the periosteum from the shaft of an affected long bone, working in the direction of an exostotically modified juxtaepiphysial area, it is found that the periosteum of the unaffected part of the shaft continues over the exostotic area. It is considerably thicker there and merges with the wall of the bursa if one is present. If no bursa is present, the periosteum is more closely adherent over the exostotic area than elsewhere on the bone, dipping in and out in such a way as to cover snugly the various prominences and depressions, also tending to fill in the latter. After it is removed, one can see clearly that the knobby surface of the affected region is cartilage-capped in some places and osseous in others, and that even the tubular projections are cartilage-capped at least at the tips, though the surfaces of their stems may be entirely osseous. One should note that in general the younger the subject the greater the proportion of the affected area that is likely still to be covered by cartilage and that in a mature subject this area may show only tiny sparse islands of cartilage if any.

When the bone is sectioned, it will be seen that the outline of the surface in the affected area coincides with the periphery of the bone. That is, the exostotic area is not superposed on a delimiting cortex but instead the outline of the exostotic area represents irregularly outpouched cortex. In the places where this cortex is still capped by cartilage the latter may vary in thickness between about 2 and about 5 mm. even from place to place in the same exostotic area. Where the outpouched cortex is solely osseous, it is a very thin plate of bone. Beneath the cortical surface the bulged-out modified area is composed of spongy bone whose trabeculae are delicate and are interspersed with considerable marrow, which on the whole is mainly fatty marrow.

If the bone in question has come from a young subject, it can be noted that the centers of ossification for its epiphyses have apparently been growing normally and that the epiphyses themselves are fairly normal in shape. The epiphysial cartilage plates per se tend to show no abnormalities. The chronology of fusion of the epiphyses with the shafts seems to be fairly normal. So also is the chronology of ossification and of fusion of the epiphyses elsewhere than in the long bones.

Microscopic Pathology of an Exostotic Area.—In those parts of the area where there is a cartilaginous surface it can be seen that the cartilage is hyaline. It may be relatively thick and may show on its inner surface considerable growth activity in the form of active endochondral ossification. If it does, the deeper-lying cartilage cells will be found lined up in columns and in general going through the gamut of changes associated with such ossification. In these areas the subchondral spongiosa is rather likely to show congested marrow spaces and some myeloid marrow. Where the cartilage is not proliferating, columnar arrangement of its deeper cells is usually not apparent and the cartilage will be seen resting on a thin plate of bone which is continuous with the plate of bone outlining the surface of the exostotic area where this surface is devoid of cartilage (fig. 3 *A*).

As already noted, the periosteum covering the surface of the affected area is continuous with the periosteum over the unaffected areas. Where it runs over prominent cartilaginous surfaces it appears as a thin, rather poorly cellular, highly

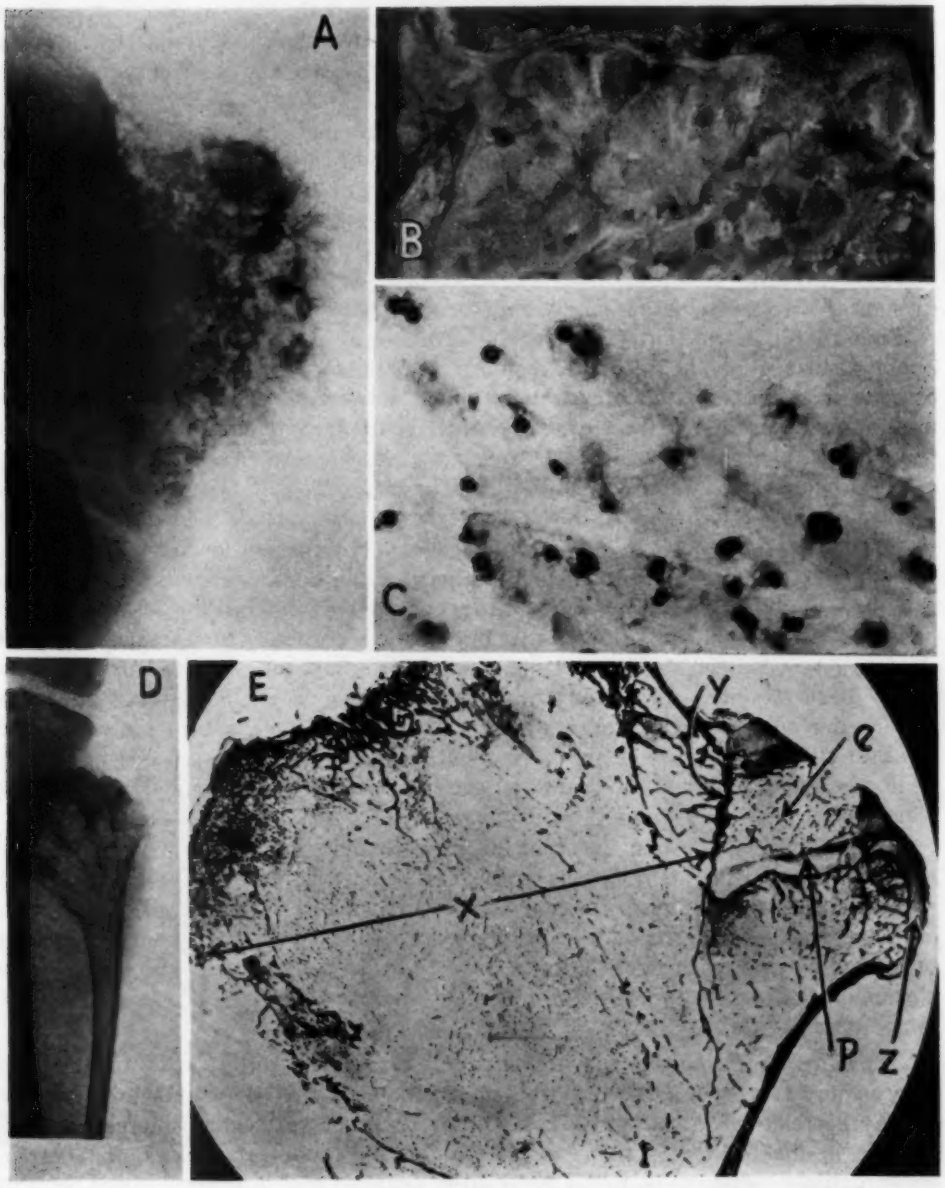

Figure 4

(See legend on opposite page)

collagenous connective tissue layer corresponding to the outer fibrous coat of periosteum in general (fig. 5 *B*). In areas not capped by cartilage there is also a relatively thick inner cellular zone which corresponds to the cambium layer of periosteum in general, but this layer is remarkable on account of its thickness.

If one searches different microscopic fields, one can find evidence of focal cartilaginous metaplasia occurring in this cellular, cambium-like layer of the periosteum (figs. 5 *C* and *D*).

One additional point should be noted. Occasionally below the surface in an exostotic area, one sees a smaller or larger focus of gritty calcareous material (figs. 2 *C*, 3 *B* and *C*). Histologically this consists in the main of calcified (largely necrotic) cartilage, calcified acellular cartilage matrix and calcium detritus. Such a focus probably results from some local disturbance of the normal course of endochondral ossification associated with the growth of the exostotic area, so that calcified cartilage and cartilage matrix which should have been destroyed and replaced by bone have accumulated under the proliferating cartilage. The finding of such a focus should not, however, be regarded as evidence that in the exostotic area in question an enchondroma is present along with the osteocartilaginous exostosis. Indeed such gritty calcified foci originate not from the interior of the bone but from the surface of the lesions and are related to the proliferating cartilage on that surface.

CHONDROSARCOMA AS A COMPLICATION

That in cases of multiple exostosis chondrosarcoma may develop in one or another of the affected areas of bone has been known for a long time. One of the earliest detailed accounts of a case in point was given by Weber [8] in 1866.

Explanation of Figure 4

A, roentgenogram showing a recurrence (the third) of chondrosarcoma. The original growth apparently began in the left ilium, and this roentgenogram was taken shortly before removal of the recurrence. At this time the tumor was of thirteen years' standing. Intense calcification of the central portion of the tumorous growth is apparent.

B, photograph (reduced by one-half) showing part of the cut surface of the larger tumor mass removed from the lesion represented in *A*.

C, photomicrograph (× 250) showing the cytologic aspect of the cartilage tumor tissue removed at the time of the first recurrence of the lesion illustrated in *A* and *B*. Note the three cartilage cells with double nuclei. Such cells were apparent in all the fields examined and are already indicative of the ominousness of the lesion and the likelihood of its recurrence. There was an interval of about eight years between removal of the first and the third recurrence, during which time the gross character of the tumor tissue apparently underwent little change.

D, roentgenogram showing the strikingly expanded upper end of a fibula of a 10 year old boy who had multiple exostosis. As is not unusual with the fibula, the bulk of the outgrowth lies toward the medial side. Note the proximal epiphysis, the epiphysial cartilage plate and the exostotically distended area on the medial side, rising up to surround much of the epiphysis.

E, photomicrograph (× 2) showing in longitudinal section the expanded upper end of the fibula illustrated in *D*. Note *e*, the proximal epiphysis; *p*, the epiphysial cartilage plate, and *x*, the exostotically distended area on the medial side, still covered in part by cartilage though much of the latter had been destroyed in the course of the surgical intervention. At *y* under higher magnification the arrangement of the tissues is found to be such that it still seems possible to recognize a line of cleavage between the epiphysis and the exostotic outgrowth which has grown up around the epiphysis. At *z* there is a small exostotic bulge capped by cartilage which joins onto the cortical bone below and the epiphysial cartilage plate above. It is clear that in the region of this bulge the periosteum has not laid down a sheath of cortical bone as it should have done. The cartilage capping the bulge seems to have been deposited by the periosteum, although some would undoubtedly maintain instead that it is extruded epiphysial cartilage plate tissue. In any event it is endochondral bone growth on the under surface of such juxtaepiphysial subperiosteal cartilage that accounts for the evolvement of the bumpy outgrowths in the affected regions.

8. Weber, O.: Virchows Arch. f. path. Anat. **35**:50, 1866.

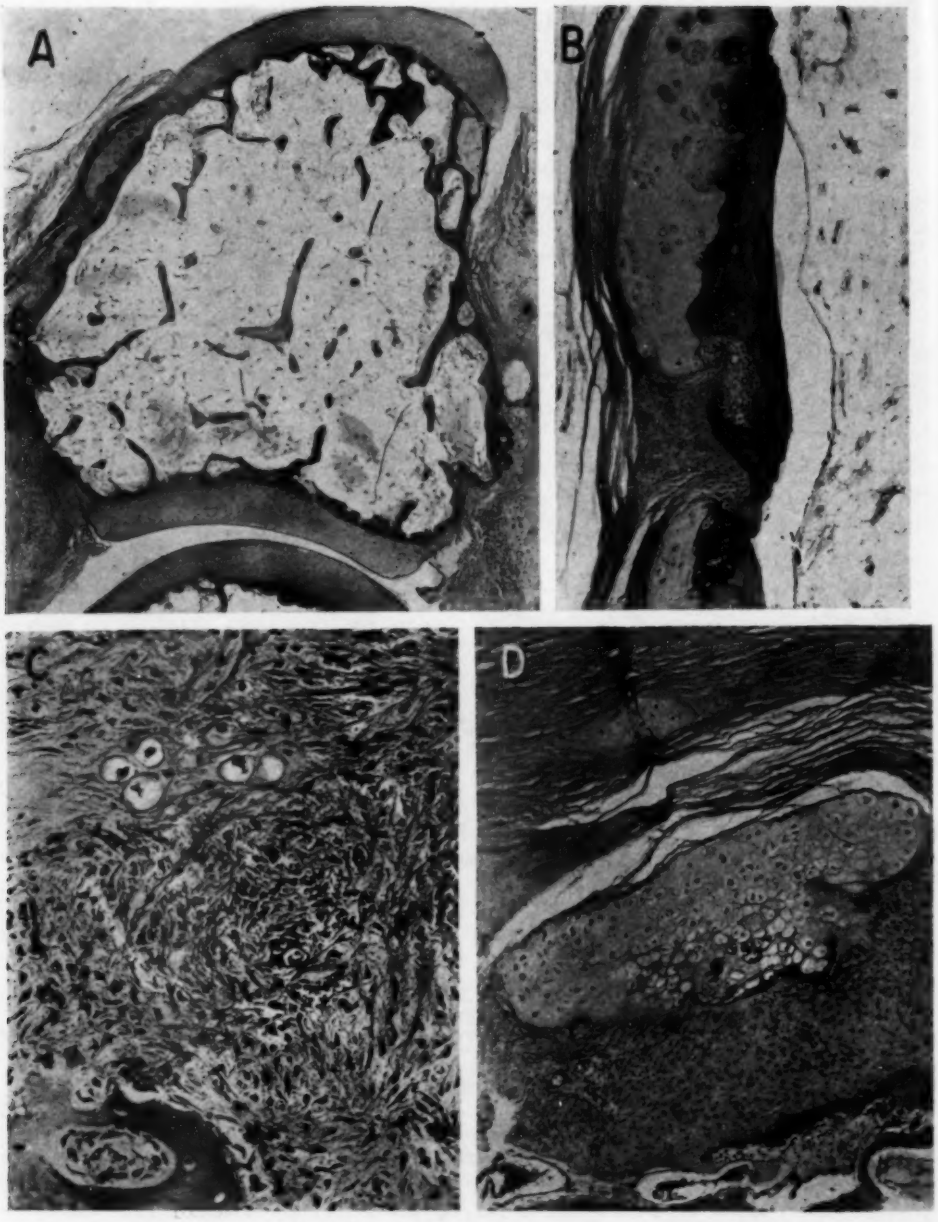

Fig. 5.—*A*, photomicrograph (× 10) showing a longitudinal section through the terminal phalanx of a toe from a boy of 15 (see fig. 1 *B*). The epiphysial cartilage plate has already disappeared, the epiphysis being fused with the shaft. In relation to the periosteum of the shaft one can note cartilage which, particularly on the surface, extends far distally.

B, photomicrograph (× 25) showing in fuller detail the appearances on the volar surface of the shaft of the phalanx illustrated in *A*. Note from left to right fibrous periosteum, resting hyaline cartilage, cortical bone and fatty marrow.

C, photomicrograph (× 125) showing some cartilage cells in the cambium layer of the thick periosteal coat covering an outgrowth on the femur of a boy with multiple exostosis. Isolated small groups of cartilage cells and even well formed bits of cartilage (see *D*) are likely to be found in relation to the periosteal coats of exostoses, especially in places where the surface of the exostotic area does not have an actual cap of cartilage.

D, photomicrograph (× 60) showing a well formed island of cartilage in relation to the periosteal covering of an exostosis, again on the femur of a boy with multiple lesions.

Numerous other accounts can be found scattered through the relevant literature to date, and most of these, too, take the form of individual case reports.

Chondrosarcoma appeared in 3 of the present 28 cases (nearly 11 per cent), but this probably does not represent the true incidence of this complication in this series of cases. The subjects were 25, 28 and 36 years old, respectively, when the malignant growths began to be manifest. Since the great majority of the other patients in this series were still children or adolescents, one must allow for the possibility that later in life chondrosarcoma might develop in some of them, too. Thus the full returns on the incidence of the complication in this series will not be in for a long time (theoretically not until all the subjects have died) and the incidence will almost certainly be much higher than 11 per cent. Though there can be no doubt that most of the patients are adults (and that often they are not even young) when chondrosarcoma appears, yet children and adolescents sometimes show this complication. For instance, the patient whose case was described by Bennett and Berkheimer [9] was 10 years old, and the one whose case was described by Chiari [2c] was 19.

It appears that chondrosarcoma may evolve out of the cartilaginous cap of one or another of the exostoses or out of the residuum of this cap. It may conceivably also evolve out of a mere cartilage rest which has been lying between the periosteum and the cortex of the affected area of bone. The tumor may grow rapidly to large size, soon break into regional venous channels, reach the heart and the lungs by intravascular growth and even give rise to parenchymal metastases, at least in the lungs. Death may ensue in the course of five or six months after the appearance of chondrosarcoma in such instances, but so rapid a course is exceptional and was not shown in any of the cases in the present series. A course leading to death within a few years is more common. Furthermore, a torpid course of ten years or more is not at all unusual. In the cases of slower evolution the cancer may remain only locally invasive for years, without spreading distantly through the venous channels, and indeed death may take place from some cause only accidentally connected with the tumor, without its having spread distantly at all.

In one of the cases in the present series chondrosarcoma had started in the left sixth rib.[10] The patient was a man who had been aware for two years of a mass on the left side of his chest. He dated the appearance of this mass from a blow which he had received six months earlier in the general region of the tumor. Along with his multiple exostoses he presented an almost grapefruit-sized tumor in the midaxillary line of the side of the chest in question. Surgical exploration showed that the mass was overhanging and separating the adjacent ribs—namely, the fifth and the seventh. It was also found that anteriorly a few small cartilaginous nodules had penetrated the capsule and were lying separate from the main mass and that posteriorly the tumor had penetrated the pleura and was growing into it. The mass was resected (though not in one piece), and its gross appearance was found rather typical of chondrosarcoma complicating multiple exostosis, at least in the earlier stages. Specifically, when it was reconstructed it was found characterized anatomically by a wide irregular outer zone of cartilage, clearly separated in some places by a line of endochondral ossification from the interior portion, which was heavily strewn with foci of bone and calcification, interspersed with islands of cartilage.

Within four months after removal of the original growth the patient showed a local recurrence, and a wider local resection of the wall of the chest was done at another institution. The tumor cartilage removed at this operation no longer showed much new bone or foci of heavy calcification such as were typically prominent in the original specimen. Several months later there was again evidence of a recurrence. The patient died almost two years after the original operative intervention and four years after he had first noted the presence of the tumor.

At autopsy, considerable gelatinous tumor cartilage was found beneath the soft tissues of the wall of the chest, and adherent to the pleura there was a mass of the same type of tissue,

9. Bennett, G. E., and Berkheimer, G. A.: Surgery **10**:781, 1941.

10. This case has already been commented on in another connection.[11a]

practically filling the entire pleural cavity on the affected side and encasing and collapsing the left lung but not extending into it. The right lung was free of tumor, and there were no distant visceral metastases.

In another of the cases chondrosarcoma had started in the left iliac bone. It ran a much more torpid course (one of thirteen years), and then the case terminated fatally only on account of a pulmonary embolism following the surgical removal of the third local recurrence of the growth. The patient dated his difficulty from a severe trauma to the left pelvic region in 1925, after which a rapidly growing but painleess mass developed in the left buttock and the adjacent upper part of the thigh, attaining a large size in the course of a year. In 1926 at another hospital this tumor mass and a block of thickened and otherwise modified ilium to which it was attached were removed. Together they weighed 1,500 Gm. The resected piece of the ilium, measuring 4 cm. in thickness, was composed of intermingled osseous tissue and cartilage. The adherent tumor mass was multinodular and consisted almost solely of large facets of cartilage which was hyaline in some places, gelatinous and somewhat cystic in others and clearly calcareous in still others.

In 1930 the patient again noticed a rapidly growing lump appearing in the same region, and not long thereafter he entered the Hospital for Joint Diseases for removal of the recurrent growth. At that time the surgeon extirpated a mass of cartilage which was lying under the fascia but was not adherent to the bone. The mass was covered by a fibrous capsule and measured 12 by 8 by 6 cm. It was composed of discrete lobules of hyaline and somewhat mucoid cartilage, again presenting areas of calcification. In 1932 a second recurrence appeared in the form of two discrete but somewhat smaller tumor masses, one of which was located in the gluteal region and the other in the region of the greater trochanter. At operative intervention for the second recurrence the tumor masses were again found not attached to the bone, and again both were composed of hyaline and mucoid cartilage showing calcareous impregnation in some places. Once more there was a prompt recurrence (the third) in the form of two discrete growths in the area in question. They grew relatively slowly until 1936, when the patient fell about 16 feet and suffered an injury to the affected area, after which the tumors took on a spurt of growth (fig. 4 *A*).

When they were removed, in 1938, they measured 20 by 15 by 14 cm. and 8 by 7 by 5 cm. respectively. Both tumor masses were apparently completely extraosseous, though in the course of liberating them a small area of the capsule of each mass was found intimately attached to the periosteum of the underlying ilium. Again, grossly the tumor was found composed of lobules of cartilage, hyaline for the most part but quite edematous and even cystic in some places and calcareous everywhere but particularly in its central portions (fig. 4 *B*).

Ten days after this last surgical intervention the patient suddenly collapsed and died. The autopsy revealed a pulmonary embolus. There were no pulmonary or other distant metastases. The large veins draining the left side of the pelvis were likewise free of tumor tissue, and no additional tumor tissue could be found remaining at the site of the surgical intervention or indeed in the pelvis either. (The autopsy confirmed, of course, the presence of multiple exostoses, already recognized clinically.) Thus over a period of thirteen years chondrosarcoma remained only locally invasive, though it recurred three times after it had originally been excised.

In the third case the course is not known to the end, contact with the patient having been lost. The patient was a woman, and chondrosarcoma had started in the sacrum, apparently without having been preceded by any special trauma. She was under observation for about two years, and in the course of this time the tumor increased tremendously in size. It was clear that its site was such as to make complete extirpation impracticable. In two attempts to remove as much of it as possible, material was obtained for anatomic study.

In the literature the cancerous tumor which develops in association with lesions of multiple exostosis is by no means uniformly referred to as chondrosarcoma or even as "malignant chondroma." Indeed, it is often denoted with unfortunate vagueness as chondroma or enchondroma, albeit large, recurrent or otherwise peculiar. Again, it may be found referred to as osteochondrosarcoma (chondro-osteosarcoma) or even, altogether incorrectly, as osteogenic sarcoma. The latter designation for the cancer complicating cases of multiple exostosis is particularly to be deplored, since there are deep-rooted clinical and anatomic differences between osteogenic sarcoma and chondrosarcoma.

In passing on to the histology it is to be noted first that unless one is aware of the cytologic peculiarities of cancerous cartilage growths, it is not unlikely that chondrosarcoma complicating multiple exostosis will be "underdiagnosed," at

least at first. As was pointed out elsewhere,[11] if a cartilage growth in relation to a bone presents, even if only in scattered areas, many microscopic fields showing several or more binuclear cartilage cells or many cartilage cells with plump nuclei, especially any cartilage cells containing large or multiple nuclei, the growth is no longer clearly benign, though other areas may look innocent (fig. 4 *C*). Subsequently, the histologic picture in such a lesion may come to be that of crudely obvious chondrosarcoma. In the second case cited (the one presenting three recurrences in the course of thirteen years) microscopic study of the original growth and the subsequent recurrences revealed on this basis some increase in ominousness of the lesion from specimen to specimen. On the other hand, the cytologic aspect of even the fourth specimen in this case was not as ominous as that of the original specimen in the first case cited. In that case also, however, the cytologic picture was more crudely sarcomatous in the recurrences than in the original lesion.

PATHOGENESIS

There are several different interpretations which have been proposed to explain the processes by which the lesions of multiple exostosis develop. For a long time the dominant conception was the one given prestige by Virchow,[12] who derived cartilage-covered exostoses on bones (as well as cartilage islands within bones—i. e., enchondroma) from epiphysial cartilage plates or their analogues. Specifically, in regard to exostoses he held that bits of cartilage become snared off from the lateral margins of the plates, and, moving on to the body of the bone, constitute the beginnings of the lesions.

It should be pointed out, however, that Virchow did not reach his views on the origin of the cartilage giving rise to exostoses (and enchondromas) from direct observations of such lesions in their formative stage. Instead, he derived his conception by deduction from conditions obtaining in rickets, in which, it is true, the juxtaepiphysial regions, particularly of long bone shafts, not uncommonly show bits of cartilage which apparently had been snared off and isolated from the plates. Altogether, the idea that the characteristic lesions of multiple exostosis start as bits of cartilage snared from epiphysial plates (or their analogues) seems inadequate per se to explain the pathogenesis.

Another conception of the pathogenesis is that the lesions have their beginnings in cartilage derived from the periosteum. This is also an old conception, but it has been gaining ground lately. Müller [7] in 1913 surveyed the literature relating to both the epiphysial plate theory and the periosteal theory, weighed them against each other and, adding evidence from the autopsy findings in a case of his own, gave impetus to the contemporary trend in favor of the periosteal mode of origin.

From the findings in his case he concluded that cartilage-covered exostoses on the bones have their beginnings in small nests of cartilage formed by the osteogenic layer (cambium layer) of the periosteum and that the small cartilaginous nodules (ecchondrosis) found on the costal cartilages and sometimes on the cartilage-preformed base of the skull originate from the perichondrium. Furthermore, he held that in this disorder there was an abnormal capacity of the periosteum and perichondrium to form cartilage during the entire period of growth and even beyond it and that this represented a perverted activity of these tissues under the influence of some constitutional aberration.

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11. Lichtenstein, L., and Jaffe, H. L.: (*a*) Am. J. Path. **19**:553, 1943; (*b*) Arch. Surg. **46**:480, 1943.

12. Virchow, R.: Berl. klin. Wchnschr., 1891, p. 1082.

In connection with the periosteal theory of origin, it should be remembered that Müller's material in itself was not of a kind which could establish it beyond doubt. Specifically, the patient was a man of 26 years, by which age the disorder has usually already fully evolved, so that deductions as to the pathogenesis are no longer entirely justifiable on the basis of the anatomic findings. Scherer [13] in 1928 supported Müller in his interpretation, again unfortunately on the basis of findings in an adult—a man aged 31. The 2 cases in the present series in which autopsy material was available were likewise those of adults and hence had the same limitations.

I come now to the conception expounded by Keith.[3] Pointing out the well established fact that normally at growth zones (epiphysial cartilage plates and their analogues) a core of bone formed by endochondral ossification becomes encased in a sheath of bone formed by the ring of periosteum surrounding the growth zone, he maintained that the primary basis of the genesis of multiple exostosis is defectiveness of this periosteal ring. In regard to the disorder in question Keith maintained that on account of the deficiency or absence of deposition of bone by the periosteal ring the cartilage-preformed bone at the growth zone is free to expand in abnormal directions, so that the normal confining and modeling at that zone fails to take place. Thus the new bone formed at the growth zone, not being restrained and subjected to active remodeling, "spills out," as it were, onto the outer surface of the body of the bone, producing the cartilage-covered excrescences. The latter, according to Keith, are thus merely the secondary results of the primary disorder of bone growth and especially bone modeling which he denotes in the name "diaphysial aclasis."

This theory has a dynamic quality about it which is very intriguing. As Keith himself concedes, however, his conception does not rest on a foundation of microscopic study of the crucial areas in question. Indeed, the material on which he founded his theory consisted only of roentgenograms of bones of a few subjects near or at the end of the period of growth and some museum specimens from the skeleton of a subject about 30 years old, and it thus lacks the evidence which would be necessary to establish it firmly.

Ideal material for the study of the genesis of this disorder would be represented by whole affected bones with evolving lesions from children (especially young ones), and strategic studies of such material have apparently not yet been reported. Nevertheless, the material available to me—and specifically the many surgically extirpated lesions from affected bones of children—was able to supply a basis for reevaluation of the various conceptions.

For instance, in one of several specimens from the upper ends of fibulas (figs. 2 *D* and 4 *E*) the appearances were such as to favor that part of Keith's hypothesis which maintains that where the exostotic outgrowths appear, in juxtaepiphysial regions, there has been a lack of cortical bone formation by the periosteal ring such as normally occurs in these regions and guides the normal shaping or modeling of the part. While accepting this part of Keith's theory, the findings from this specimen permit one to hold at the same time that perverted activity of the periosteum is also a factor in the genesis of the lesions. This idea, which is in line with Müller's, would mean that precisely in the juxtaepiphysial regions periosteal activity is disordered or perverted on a deeply rooted hereditary basis and specifically that the periosteum is forming cartilage instead of bone.

In many of the other specimens examined by me evidence that the periosteal covering of the lesions does possess the capacity to form islands of cartilage in this

13. Scherer, E.: Frankfurt. Ztschr. f. Path. **36**:587, 1928.

disorder could be demonstrated (fig. 5). Furthermore, the inculpation of the coverings of bones and cartilages would explain the presence of nests of cartilage in the perichondrium (ecchondrosis) such as one often observes on the costal cartilages in these cases. It would likewise account for the occasional occurrence of an excrescence on one or another of the bones at the base of the skull, which are preformed entirely in cartilage. Also, it would harmonize with the occasional finding of a large cartilage-covered exostosis on the body of a rib near the mid-axillary line when the shaft of the rib near the costochondral junction is relatively well modeled, showing perhaps only a wreath of tiny nodular excrescences.

Altogether, on the basis of histologic study of material such as could shed some light on the question I hold that the theory of defective modeling and that of perverted periosteal and perichondral activity are not only mutually compatible but reenforce each other in explaining the genesis of the disorder as a whole.

INHERITANCE

The recognition of an inheritance factor in the occurrence of multiple exostosis dates clearly from the description by Stanley, in 1849, of a family manifesting the disorder in three generations. Between that time and the present numerous observers have collected and recorded pedigrees of persons presenting the condition, to emphasize this aspect of it. Quite the fullest and most comprehensive presentation of the hereditary features of the disorder is the one by Stocks and Barrington,[14] published in 1925. Their analysis is based on practically the full sum of the cases reported up to that time, and they also give a full bibliography. Incidentally, it should be pointed out that in their study, as in too many other studies both before and after theirs, multiple exostosis and multiple enchondromatosis are linked as closely allied disorders, regarded as often appearing in the same person or in the same family. However, this does not vitiate their findings since in their statistical analyses the "pure" cases of multiple exostosis are kept separate from those supposedly also showing enchondromatosis.

Specifically, Stocks and Barrington analyzed 1,124 such recorded cases of multiple exostosis. The deep-rootedness of the hereditary factor[15] is clearly revealed in the finding that 727 of these cases came from only 163 family trees. Thus in 64 per cent of the 1,124 cases the disorder was definitely not limited to one member of a family tree but occurred also in one or more antecedents or relatives. In the remaining 36 per cent (the other 397 cases) it was found that either the relevant information about the family tree was vague or missing or else that the records definitely stated that no other member of the subject's family was known to be affected. In the latter group of cases—that is, those with a definitely negative family history—the possibility must be considered that the disorder represents a mutation in the affected subject. However, this postulate is safe only if one can be certain that the disorder was absent not only in the immediate family of the affected subject but also for at least two generations back.

Returning to the cases in affected family trees—that is, the clearly inherited cases—Stocks and Barrington found that in somewhat more than half (about 60

14. Stocks, P., and Barrington, A.: Hereditary Disorders of Bone Development, University of London, Francis Galton Laboratory for National Eugenics, Eugenics Laboratory Memoir 22, 1925.

15. An instance of the disorder in a pair of monozygous twins has also been reported (Birkenfeld, W.: Zur Erblichkeit der multiplen cartilaginären Exostosen [Exostosen bei eineiigen Zwillingen], Deutsche Ztschr. f. Chir. **226**:397, 1930) Another instance of the occurrence of the disorder in twins is cited by Stocks and Barrington.[14]

per cent) the condition could be traced directly from an affected parent to an affected child. The parent in question was the father in 73 per cent and the mother in only 27 per cent of these directly inherited cases. It should be noted, however, because of its practical significance, that an unaffected male in a family tree in which the disorder is prevalent apparently does not transmit it to the children. On the other hand, an unaffected female in such a family tree may do so, for it appears that in the female the disorder may be latent or suppressed. Indeed, this latency seems to be not uncommon, apparently existing in about 1 of every 4 females who transmit the disorder to their children, as indicated by the presence of the disorder on the mother's side of the family. Furthermore, the proportion of children affected is just as great when the condition is transmitted through an unaffected as when it comes through an affected mother.

It may be expected that about one half of the offspring in families in which one parent has multiple exostosis will show the condition. Affected males hand on the disorder to about 52 per cent of their children, and affected or tainted females, to about 42 per cent. It appears, moreover, that in the course of successive male transmissions the percentage of children affected rises sharply above 52. As to position in the family, the elder-born members of exostotic stock are more likely to be affected than the younger children. As to sex distribution, the disorder shows, as already noted, a definite predilection for males, for when large numbers of cases are considered, about 70 per cent of the patients are found to be males and only about 30 per cent females. One reason for this preponderance of males among the subjects is that, altogether, deviations from the norm tend to be more common in males than in females. In addition, however, as already pointed out, a considerable proportion of unaffected females in families showing the disorder are yet able to transmit the disease. It is the fact that these latent cases do not show up to be counted as instances of the disorder that is mainly responsible for the relatively low incidence in females as compared with males.

Certain observations on inheritance have been made also in regard to the number and the distribution of the exostoses themselves. All in all, the offspring of subjects with relatively few exostoses tend to have relatively few, while the offspring of subjects with numerous exostoses tend to have large numbers. However, in any affected family tree the exostoses tend to become more numerous from generation to generation.

SUPPOSED INTERRELATIONS BETWEEN MULTIPLE EXOSTOSIS AND ENCHONDROMATOSIS

As was already mentioned in connection with nomenclature, a common current opinion is that multiple exostosis and enchondromatosis represent manifestations of a general "osteogenic disease" which (1) may express itself in either multiple exostoses or multiple enchondroma (even in different subjects of the same family tree) and (2) is also commonly manifested in the appearance of both disorders in the same person. For many years I have been trying to ascertain whether there was actually a clinical and pathologic foundation for this conception, but I have not been able to confirm the existence of the type of interrelationship indicated here.

First of all, in the 28 cases which form the basis of this study of multiple exostosis there was never any evidence of enchondromatosis in the family of the persons affected, but instead, when any other member of the family was known to have any sort of similar generalized skeletal disorder, it was always skeletal exostosis. (Conversely, among the cases, far fewer, of skeletal enchondromatosis studied there were no instances of multiple exostosis in the family of the patient,

nor was there even skeletal enchondromatosis in other members of the family.) In the second place, in my own material I found no anatomic basis directly linking the two disorders. Nevertheless, it is not difficult to discover by a critical survey of the literature some of the sources of the confusion between cases of enchondromatosis and multiple exostosis.

Such a survey will show, for instance, that a great many authors loosely refer to the peripheral cartilaginous caps of the cancellous exostoses as "enchondromata" in spite of the fact that etymologically the word "enchondroma" should be limited to a cartilage mass definitely involving the interior of a bone. As to the focus of calcified cartilage sometimes seen at the periphery of an exostotic area (figs. 2 *C* and 3 *B* and *C*), it has already been pointed out that such a focus does not represent anything akin to enchondroma. Instead, as noted, it represents merely an area of calcified cartilage which has accumulated in consequence of a localized disturbance of endochondral ossification of the cartilage cap in an exostotic area. Nowhere does one find in the exostotic lesions frank masses of cartilage spreading out from the interior of the bone and distending its surface. In contrast, the lesions of skeletal enchondromatosis are composed essentially of such masses, which, especially in the bones of the hands and feet, may widely distend the cortex and make its contour irregularly bumpy by bulging from within. Again, chondrosarcoma developing out of the cartilaginous cap of one of the cartilage-covered exostoses is sometimes loosely denoted as "malignant chondroma" or even "malignant enchondroma." Finally, some confusion has resulted from incorrect interpretation of roentgenographic appearances. Often, if the affected area is roentgenographed in one plane only, an exostosis will not show up as a projection from the cortex. Instead, it will cast a shadow revealing only the outline of its margin where it extends out from the cortex, and the outlined area may thus appear as though framed within the bone and be misinterpreted as enchondroma.

Altogether, I think enough has now been said to make it clear that multiple exostosis and skeletal enchondromatosis should be regarded as clinically and anatomically independent disorders.

COMMENTS ON SOLITARY EXOSTOSIS (SOLITARY OSTEOCHONDROMA)

The so-called solitary osteochondroma or solitary osteocartilaginous exostosis is the commonest of the tumorous lesions encountered in bone. The records of the laboratory of our hospital include at least 150 cases, and the present comments are based on a resurvey of 50 consecutive ones in which material from the lesions was received between February 1939 and July 1942.

Like the lesions in cases of multiple exostosis, the solitary exostosis develops in relation to a metaphysial or in any event a juxtaepiphysial region of bone. Though it may appear on such an area in practically any bone, there is no doubt that it definitely predilects the lower metaphysis of the femur and the upper metaphysis of the tibia. It simulates the individual lesions of multiple exostosis not only in regard to localization but also in regard to roentgenographic and anatomic appearances, both gross and microscopic. Indeed, it seems clearly to represent the localized one bone analogue of multiple exostosis, though on the whole less exuberant even as a single lesion. Its pathogenesis is probably the same as that for the individual lesions in cases of multiple exostosis. As to etiology, while for the latter one can inculpate a deep-rooted hereditary defect of skeletal formation, the influence of a hereditary factor has not been clearly established in cases of solitary osteochondroma.

TREATMENT

The mere presence of multiple exostosis in a subject is not in itself a reason for intervention against the lesions. However, removal of one or another of the bony prominences may be sought for cosmetic reasons or on account of local pain. It may also be indicated when a lesion has resulted in interference with the movement of a joint or given rise directly or indirectly to some gross skeletal deformity. The choice of procedure is, of course, a matter for the surgeon and will be governed by the aim to be accomplished in a given case. In general, when exostotic areas are excised, the periosteum should be removed with them and not stripped back. That is, the excision should not be subperiosteal since remaining periosteum may invite recurrence.

The management of an affected area of bone which has undergone chondrosarcomatous transformation is likely to try surgical judgment. In general, the surgical decision should lean toward the radical side. Fortunately, chondrosarcoma (at least in easily accessible areas such as limbs) does not offer the gloomy prognosis of osteogenic sarcoma, provided that amputation is done well beyond the level of the lesion. In sites such as the pelvis, in which thorough eradication of the affected area is surgically difficult and may even be unfeasible, one may have to be content with resection, though the results of this are likely to be unsatisfactory in the long run. In regard to the value of radiation for chondrosarcoma it has become clear that this has no therapeutic effect on the course of the growth.

Finally there are two more points which seem worth mentioning. In a woman with multiple exostosis it appears advisable to roentgenograph the pelvis in case there should be a large exostosis which might act as an obstruction during childbirth. An adult with multiple exostosis should be informed that the condition will be likely to reappear in about half of his or her offspring.

SUMMARY

Hereditary multiple exostosis is a congenital anomaly of skeletal development. The lesions tend toward bilateral and symmetric distribution and appear in juxtaepiphysial areas. There is a positive relation between the severity of involvement of a particular juxtaepiphysial part and the activity of skeletal growth in that area. The involvement of the long tubular bones (especially of the lower limbs) is more marked than that of the trunk bones. Peculiar curvature and deformity of the forearm frequently develop in this disorder. Chondrosarcomatous transformation of one or another of the exostotic areas is not uncommon, particularly in adult subjects, having occurred in 3 of 28 cases.

The exostotic area is not superposed on a delimiting cortex but represents an outpouching of the cortex of the affected region. Where the lesion is growing, the surface of the outpouched area presents a zone of hyaline cartilage at least several millimeters thick, with active endochondral ossification on its under side. Where the lesion is no longer growing, there may be only a narrow zone of nonproliferating cartilage which rests on a thin plate of bone, or the surface may consist merely of a thin plate of bone not capped by cartilage. The tissue beneath the cortical cap is spongy bone in which the trabeculae tend to be delicate and are interspersed with marrow, which on the whole is fatty marrow. Islands of calcified cartilage may accumulate locally because endochondral ossification of the cartilage cap has been disturbed in the area. Such a focus does not represent enchondroma.

As to the genesis of multiple exostosis, Keith's theory of defective modeling of the bone (diaphysial aclasis) and the conception of perverted periosteal and perichondral activity not only are mutually compatible but reenforce each other in explaining the evolution of the lesions. As to the hereditary aspects, about half of the offspring of an affected subject come to manifest the disorder. The disorder may be transmitted through unaffected females in a family tree—a fact which explains in part the excess of affected males over affected females (in a ratio of about 7:3).

As to treatment, the presence of multiple exostosis is not in itself a reason for intervention against the lesions, but when an exostotic area is excised because of some local difficulty that it is causing, it should be removed intact, with its fibrous periosteal coat, as a precaution against recurrence.

GRANULOMATOUS PROSTATITIS

A HISTOLOGIC STUDY OF A GROUP OF GRANULOMATOUS LESIONS COLLECTED FROM PROSTATE GLANDS

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Our purpose in the study which gave rise to this article was to become acquainted with the histologic appearance of an unusual lesion in the prostate gland resembling granulomatous changes to investigate its genesis and finally to determine its cause as accurately as could be done by almost purely histologic means. Bacteriologic and serologic methods were used in the few cases in which it was possible to employ them, and these methods, together with a brief review of the clinical findings, aided in the evaluation of certain etiologic possibilities.

This chronic inflammatory lesion had been noticed occasionally in surgically removed prostatic tissue over a period of years at the Mayo Clinic and usually had been designated "granulomatous prostatitis." Some of the cases were suggestive of tuberculosis, syphilis or some other specific disease, but in all specimens there were some features which were similar among themselves and the exact nature of the lesion remained undetermined. It was with such a background that the study of cases was begun.

The medical literature regarding diseases of the prostate gland failed to reveal any previous studies of a group of cases such as ours. Because of this, the findings of this study will be presented first, and then the literature pertaining to various specific granulomatous lesions in the prostate gland will be cited.

In referring to the lesion described in this study, the term "granulomatous prostatitis" will be used.

MATERIAL

The cases of granulomatous prostatitis presented in this paper were selected from a large group of cases of inflammatory lesions in surgically removed prostatic tissues. Some cases previously classified as being of specific origin were reclassified and were included in this study. Of 1,028 inflammatory lesions in surgical specimens, 34 presented a picture of granulomatous inflammation and are included in this study. Of 88 inflammatory lesions in prostate glands removed at necropsy, 3 fulfilled the histologic criteria of granulomatous prostatitis and are included in this study.

Thus, 37 specimens selected from the aforementioned groups form the material for this study. The tissue is purposely mostly surgical in origin, because the problem of diagnosis of the lesion concerns surgically removed tissue much more than tissue obtained at necropsy. The 3 specimens obtained at necropsy are included for the purpose of helping to rule out certain generalized diseases or etiologic possibilities. No attempt has been made to obtain every case of granulomatous prostatitis in the records of the clinic, and this study cannot be used as the basis of a statistical analysis of the incidence of granulomatous prostatitis.

All of the laboratory and clinical data presented herein were obtained after the diagnosis of granulomatous prostatitis had been established by histologic examination. These data are included in this study only to help to eliminate, or to augment, the etiologic possibilities suggested by the histologic picture.

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DESCRIPTION OF LESION

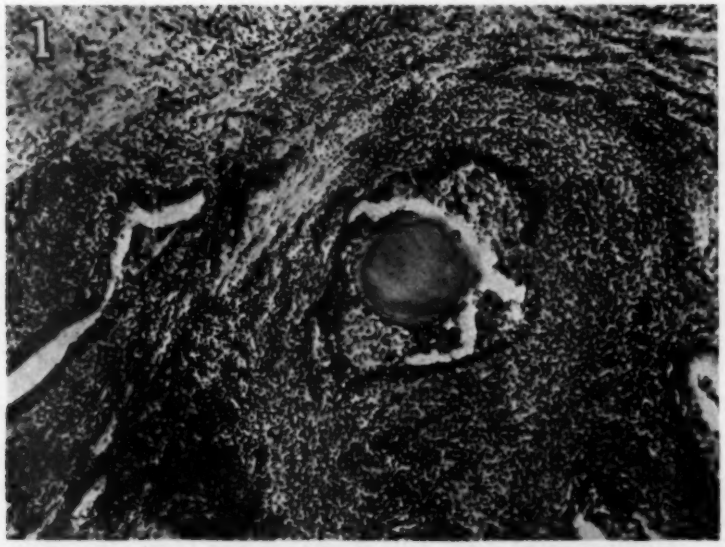
The gross appearance of the tissues was not specifically characteristic. Twenty-eight of the 37 specimens were removed by transurethral operation, and the anatomic relationships of the lesion to the gland as a whole were therefore not determinable. The remaining 9 specimens (2 removed at perineal prostatectomy, 4 at suprapubic prostatectomy and 3 at necropsy) contained small cysts or pockets, often near the prostatic portion of the urethra. These "cysts" sometimes contained caseous or purulent material, or small calculi, and probably were dilated ducts. Other portions in which changes had taken place were deep in lateral lobes and were not associated with grossly distended ducts. Tissue in these portions was firm, yellowish and finely granular. In all specimens in which the entire gland was available for study, gross enlargement due to nodular hypertrophy was present.

The general histologic appearance of the lesion in all the specimens studied was that of chronic inflammation. More specifically, it was that of granuloma. The normal acini and ducts of the prostate gland were almost completely destroyed in the regions of involvement. The acini had been replaced by a dense infiltration of lymphocytes, plasma cells and large, pale-staining mononuclear cells. In some portions there was an increase in collagenous tissue. Polymorphonuclear and eosinophilic leukocytes were occasionally present. Portions of ducts and acini could be recognized in some parts, and remnants of corpora amylacea were of frequent occurrence. Multinucleated giant cells of the foreign body type were present in some portion of nearly every specimen. Rarely did these giant cells contain crystalline or amorphous debris. Frequently, the large mononuclear cells contained what appeared to be foamy cytoplasm when the hematoxylin and eosin stain was used and intracellular lipoids when the sudan IV stain was used. Extracellular lipoid also was present in these same portions. Most of these general features are illustrated in figures 1, 2, 3 and 4.

We could not determine by purely histologic methods the presence of benign adenofibromatous hyperplasia (benign hypertrophy), although the gross findings and the clinical observations, to be given later, suggest that it was present in all cases.

The foregoing description and the illustrations briefly set forth the general character of the lesion, but in other parts of the gland the picture was less advanced, and it was from these other parts that more of the genesis of the lesion could be learned. Also, it was through an attempt to explain the genesis of this general histologic change that clues could be obtained to the nature of some of the variations which so closely resembled granuloma of syphilitic, tuberculous or other specific origin.

In every case, sections from regions somewhat removed from the diffusely granulomatous portions revealed dilated ducts and acini which were filled with polymorphonuclear cells, amorphous debris and desquamated epithelial cells. These portions were most readily found near the urethral surface, where the ductile system was most prominent. The dilatation was interpreted as evidence of obstruction of the ducts (fig. 5). In other portions of the same gland the smaller ducts were dilated and filled with similar material. The earliest evidence of a granulomatous reaction was seen around some of the smaller dilated ducts and acini, where the lining epithelium was being destroyed and where the walls of the ducts were no longer intact. Around such places there were many lymphocytes, plasma cells and large mononuclear cells. These early changes are illustrated in figures 6 and 7. The early inflammatory reaction around these "ruptured" ducts



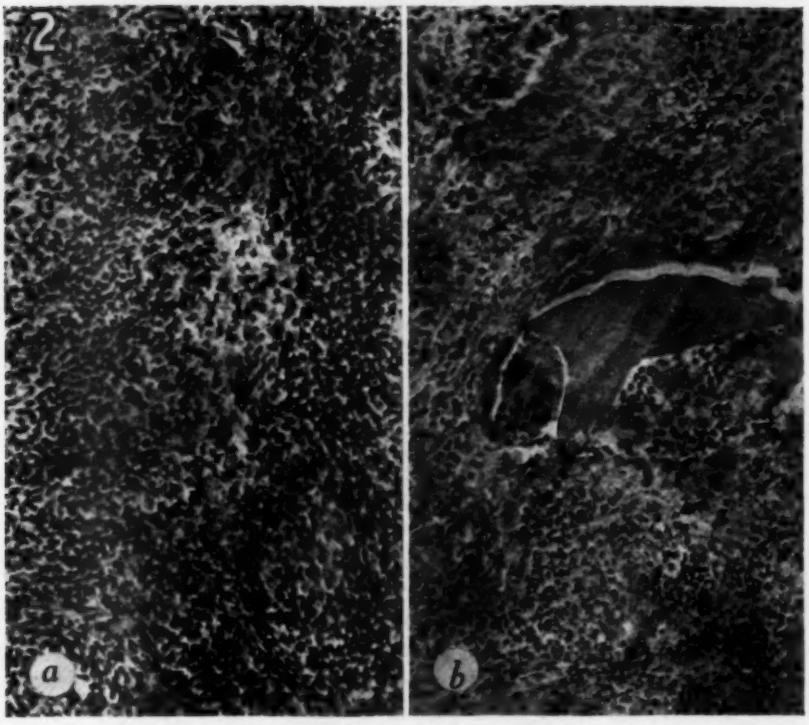
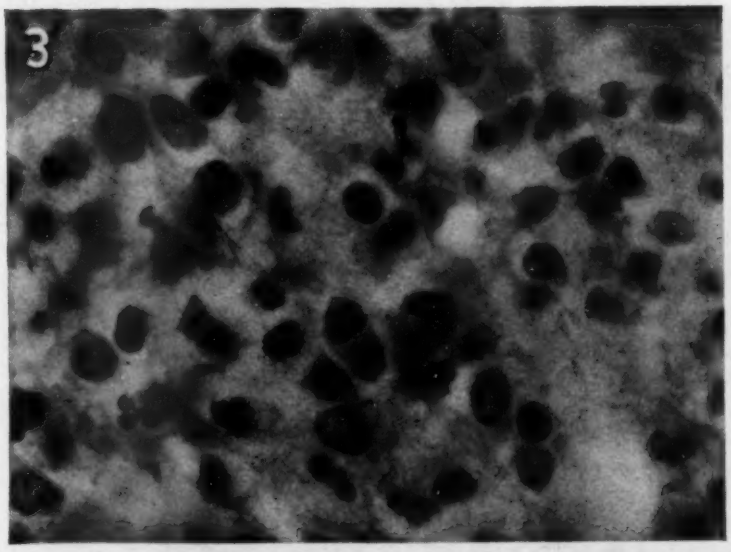


Fig. 1.—Granulomatous prostatitis with partial destruction of the parenchyma of the gland. One of the corpora amylacea is present, indicating the site of a duct. Hematoxylin and eosin; × 75.

Fig. 2.—Granulomatous prostatitis with complete destruction of the glandular parenchyma. There is dense infiltration of tissues by chronic inflammatory cells. There is a multinucleated giant cell in *b*. Hematoxylin and eosin; *a* × 135; *b* × 60.



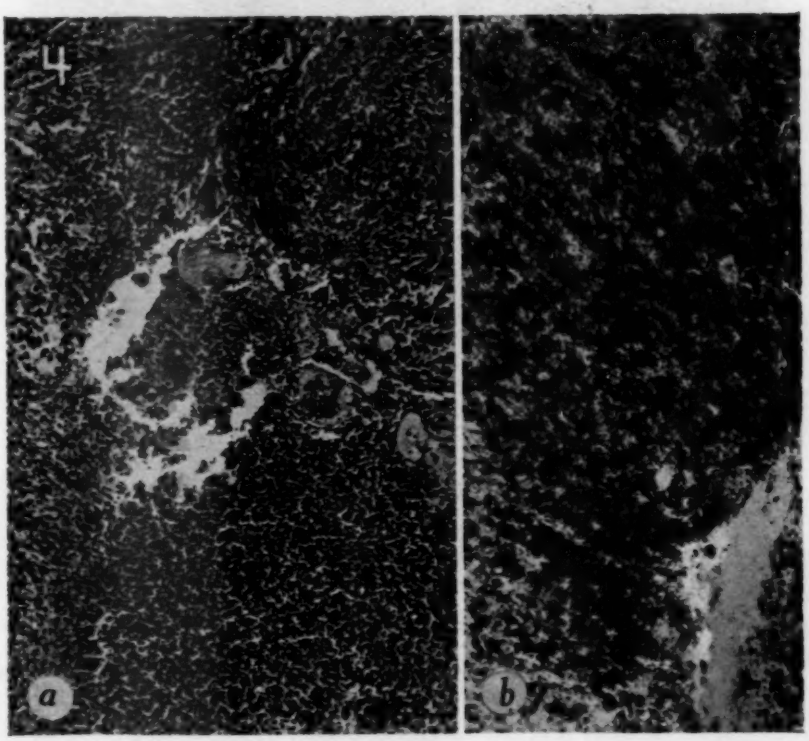


Fig. 3.—Details of the cellular reaction in granulomatous prostatitis. Note plasma cells, lymphocytes and large mononuclear cells. Hematoxylin and eosin; × 880.

Fig. 4.—Granulomatous prostatitis demonstrating foam cells; *a* hematoxylin and eosin × 65; *b* sudan IV × 115.

was of the chronic granulomatous type described as characteristic of granulomatous prostatitis. However, the contents of the lumens of the "ruptured" ducts still included a few polymorphonuclear cells plus many of the large pale-staining mononuclear cells. Apparently, then, there was a relationship between the inflam-

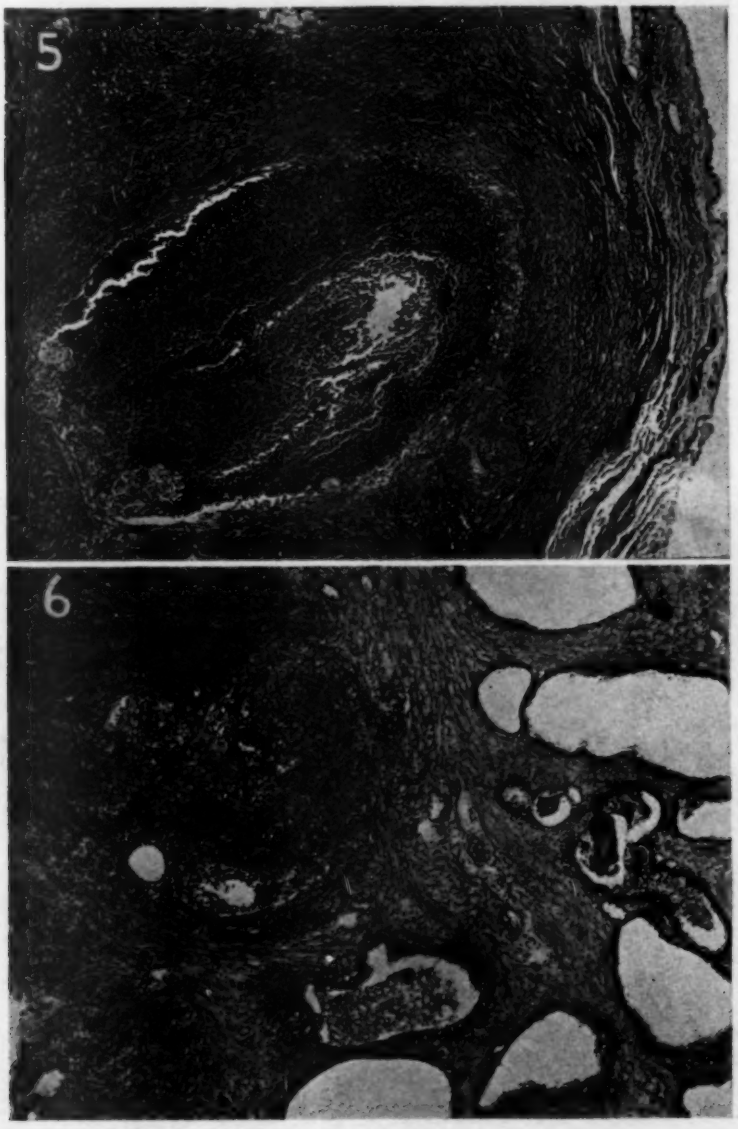
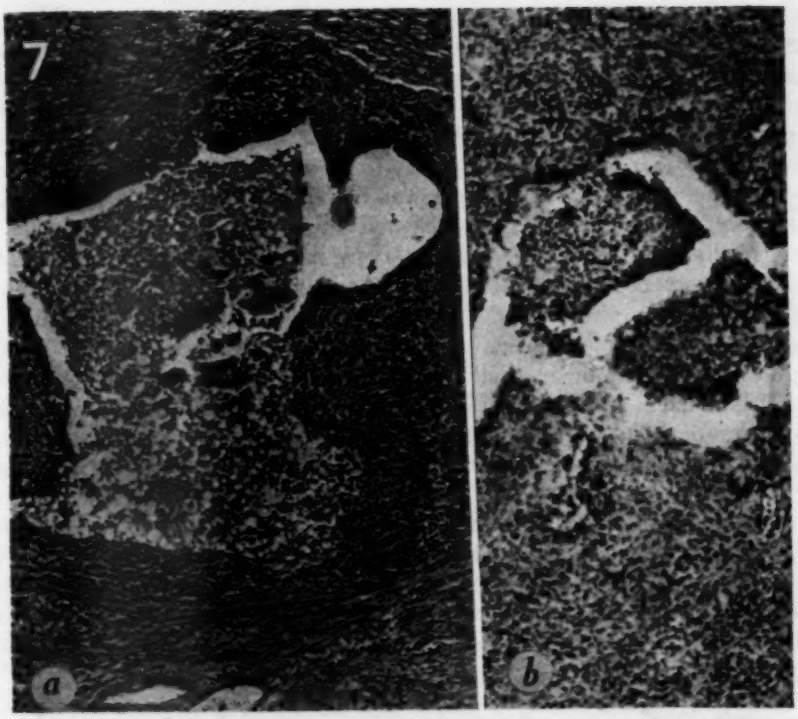


Fig. 5.—Prostatic urethra (top) with underlying dilated duct filled with inflammatory debris; hematoxylin and eosin; × 25.

Fig. 6.—Dilated acini and ducts containing a few pale-staining mononuclear cells and a few polymorphonuclear cells. Nearby is a "nodule" of granulomatous inflammatory reactions. Hematoxylin and eosin; × 45.

mation within the distended ducts and the diffuse picture of granulomatous prostatitis.



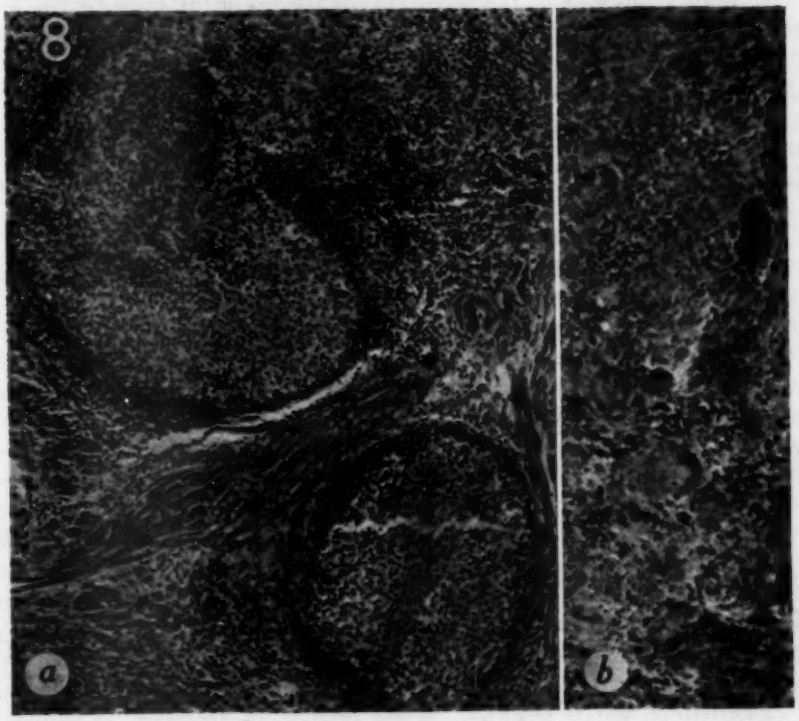


Fig. 7.—Granulomatous prostatitis in the early stage, illustrating partial destruction of the walls of prostatic ducts; hematoxylin and eosin; *a* × 65; *b* × 115.

Fig. 8.—Granulomatous prostatitis simulating tuberculosis; hematoxylin and eosin; *a* × 85; *b* × 145.

The role of cells referred to as "large pale-staining mononuclear cells" has been mentioned several times. These cells were prominent in all the specimens and particularly when a section of the tissue in which a granulomatous reaction had occurred was stained for the presence of fat (fig. 4 *b*), for these cells were seen to contain much intracellular lipoid. Cells of this type had a prominent part in some of the variations of the general histologic picture and particularly in those instances in which the lesion resembled tuberculosis of the prostate gland. Morphologically, the cells seemed to be simple large mononuclears, derived from the blood or from the tissues in the region of prostatic inflammation; however, because in the earlier phases of the granuloma the cells often appeared first intraductally and sometimes contained mitotic figures, it seemed possible that these cells arose from the lining epithelium of ducts or acini. Stains for mucus were used on 13 selected specimens in an attempt to gain evidence of the epithelial origin of these cells. Mucus was found in the region of granulomatous reaction in all cases but, because the mucus was both intracellular and extracellular and because of lack of evidence [1] that epithelial cells from the acini or small ducts always normally contain mucus, the findings did not prove these mononuclear cells to be of epithelial origin. Those mononuclear cells that did contain mucus might have acquired it by phagocytosis. The finding of mucus in all these specimens does, however, offer further evidence of the relation of this lesion to the duct system when considered in the light of Schlachta's and Pilcher's work on the presence of mucus in the normal prostate gland.

In the foregoing descriptions and illustrations of granulomatous prostatitis there have been references to the similarity of the lesion in some of the instances to tuberculosis of the prostate gland. In many of the instances the finding suggestive of tuberculosis was obviously only a phase of the general picture of granulomatous prostatitis, and in other portions of the same gland was seen the typical picture of chronic nonspecific inflammation. The picture presented in other instances, however, was certainly similar to that of tuberculosis (fig. 8). In these other instances there appeared to be discrete, circumscribed portions composed of epithelioid cells, with associated lymphocytes and giant cells. More careful study revealed, however, that the circumscribed form was due to the confining walls of ducts and that the "epithelioid" cells were actually derived from the large mononuclear cells seen in the previously described granulomatous lesion. Transition forms from the general granulomatous reactions to the picture of pseudotubercles could be found in all of these questionable cases. In these cases the inflammatory reaction appeared to have been confined within the ducts; the duct epithelium had been destroyed, just as in the other type of granulomatous lesion, but the walls of the ducts had remained intact. The evidences of more acute inflammation had vanished, and the large mononuclear cells had become small and spindle shaped and had filled the ducts. Thus, when sections of the gland were cut, certain areas had the appearance of circumscribed nodules or tubercles. The lack of any caseation or of karyorrhexis, even when the lesion was extensive, was worthy of note. The cytoplasm of giant cells in these cases frequently contained crystalline or amorphous debris. The transition, in the same specimen, of dilated ducts partially filled with large pale-staining mononuclear cells and polymorphonuclear cells to the picture of pseudotubercles without caseation was found after careful examination and is evidence of the relation of this type of lesion to the general picture of granulomatous prostatitis.

1. Pilcher, F., Jr.: Am. J. Clin. Path. **8**:366, 1938. Schlachta, J., cited by Pilcher.

The presence in the granulomatous lesion of portions wherein the infiltration was composed largely of plasma cells led to consideration of the diagnosis of syphilis of the prostate gland. The histologic picture bore only a very superficial resemblance, however, to any recognized type of syphilitic lesion. The cellular reaction was not perivascular; there was no arteritis in any of the cases, and necrosis of tissues with "ghost" forms was absent.

Another variation from the general picture of granuloma is concerned far more with clinical findings than with any histologic variation, but will be included here. In some portions of the involved gland, the epithelial elements were entirely lacking, and yet only a very few, scattered inflammatory cells were seen. The parenchyma was replaced by dense fibrous connective tissue, which undoubtedly made that portion of the gland extremely hard and might account for the clinical diagnosis of carcinoma in some of the cases. Histologically, of course, there was no confusion with carcinoma, and the portions under consideration appeared to be regions in which the more active granulomatous inflammation had subsided and proliferation of connective tissue had occurred subsequently.

In concluding the histologic description of the lesion, it is necessary to discuss procedures of which the results were negative. Because of the importance of obtaining all possible evidence favoring, or disproving, the ideas that Mycobacterium tuberculosis is the etiologic agent in many of the cases, stains for tubercle bacilli in the prostatic tissues were made in 15 specimens of the granuloma and, as controls, in 3 lesions which possessed the histologically typical characteristics of tuberculosis of the prostate gland. The usual Ziehl-Neelsen carbolfuchsin stain for tuberculi bacilli in tissues revealed no mycobacteria in the 15 cases of granulomatous prostatitis but was positive in 2 of the 3 control cases. Microscopic examination with the aid of a fluorescent dye and ultraviolet rays, originally described by Hagemann,[2] Thompson [3] and others,[4] indicated that this method was superior to the usual carbolfuchsin method for demonstrating tubercle bacilli in smears. It was considered likely that it might be applied to sections of tissue with equal success. A technic was devised, therefore, for staining sections of tissue with the fluorescent dye auramine and then examining microscopically by means of ultraviolet rays for tubercle bacilli. This procedure has been published previously,[5] and because the details are not directly related to this study, the method will not be discussed here. When ultraviolet rays and auramine were used, at all events, tubercle bacilli were not found in the specimens of granulomatous prostatitis but were found in all 3 control specimens.

Stains for Spirochaeta pallida in tissues were made in 10 of the cases in which the granulomatous lesion was most suggestive of syphilis; the method of Levaditi was employed. In none of these cases did the lesion contain spirochetal organisms.

In all the cases careful microscopic examination of the granulomatous lesion in reduced light did not reveal yeasts or yeastlike organisms. Careful examination with the oil immersion lens failed to reveal any inclusion bodies or other findings which might suggest a definite etiologic factor for the lesion.

Gram's stain for bacteria in tissues revealed a few gram-positive cocci in 4 cases and a mixture of gram-positive cocci and gram-negative bacilli in 2 additional cases. In all these 6 cases in which bacteria were demonstrated, the organ-

2. Hagemann, P. K. H.: Zentralbl. f. Bakt (Abt. 1, Beihft.) **140**:184, 1937.
3. Thompson, L.: Proc. Staff Meet., Mayo Clin. **16**:673, 1941.
4. Keller, C. J.: München. med. Wchnschr. **85**:2024, 1938. Richards, O. W., and Miller, D. K.: Am. J. Clin. Path., Tech. Supp. **5**:1, 1941. Rihl, H.: Wien. klin. Wchnschr. **53**:322, 1940.
5. Tanner, F. H.: Proc. Staff Meet., Mayo Clin. **16**:839, 1941.

isms were situated in or near the larger ducts, close to the surface of the prostatic urethra. In none of these cases in which bacteria could be demonstrated in sections of tissue were cultures of the urine positive, nor were these cases in the small group in which cultures of surgically removed tissue were made.

The final item of these negative findings deals with the 3 necropsy specimens and with 1 surgical specimen from a patient who came to necropsy ten days after operation. As to the 3 necropsy specimens, careful gross microscopic examination of the other organs of the body in each instance did not reveal the granulomatous lesion. In the single surgical case in which postmortem examination was made, a few healed lesions were revealed, resembling inflammation, at the hilus of one lung and in the pulmonary hilar lymph nodes. These were interpreted as probably old silicotic lesions. There was no granulomatous lesion similar to that found in the surgical specimen from the prostate gland in any of the other organs of the body, and even the remaining prostatic tissue failed to reveal additional sites of the granulomatous lesion.

These 4 cases are evidence that the lesion in the prostate gland is not part of a generalized bodily disease, nor even part of a diffuse genitourinary infection. Rather, it is a lesion limited to the prostate gland.

CLINICAL AND LABORATORY FINDINGS

The following facts regarding the patients represented by the surgically removed specimens were obtained only after the cases had been classed as instances of granulomatous prostatitis on a histologic basis. The clinical and laboratory findings are helpful in furnishing additional evidence for or against certain etiologic possibilities suggested by the pathologic picture. These findings are briefly summarized in the following paragraph.

The ages of the patients ranged from 22 to 89 years; only 3 patients were less than 50 years of age, and the average age was 65 years. Twenty-four patients gave a history of intermittent urinary obstruction of several months' to several years' duration; their condition was clinically typical of prostatic obstruction. Of the remaining patients, 3 had vesical calculi, 3 had chronic cystitis and prostatitis, and 2 entered the hospital with sudden urinary obstruction but gave no history of previous prostatic disease. One patient had prostatic enlargement associated with a vesical neoplasm, and another, with no complaints referable to the urinary system, was found on routine examination to have an enlarged prostate gland and residual urine. Four patients previously had undergone surgical operation on the prostate gland.

Before operation none of the patients gave any history of, or clinical evidence of, tuberculosis. Three patients gave a history of having had gonorrhea many years prior to operation. Two patients gave a history of syphilis, but none gave clinical or serologic evidence of syphilis (there was no report of serologic examination with reference to 5 patients). Six patients gave a history of having had typhoid fever early in life, and 3 of these stated that they also had had malaria. One patient had a stricture of the rectum, and the possibility of this being due to previous lymphogranuloma inguinale was considered clinically. The Frei test for lymphogranuloma inguinale was performed on 3 patients (not including the patient just mentioned) in whose cases the diagnosis of granulomatous prostatitis was made at the time of the initial pathologic examination, and in all 3 the result of the test was negative.

The preoperative clinical diagnosis in the cases of granulomatous prostatitis was "benign hypertrophy of the prostate gland" in 23 cases and "carcinoma" or

"probably carcinoma" in 9 cases. In 2 cases a preoperative diagnosis was not recorded. It may be of interest to note that the surgeon made an operative diagnosis of granulomatous prostatitis in 2 cases and of carcinoma in 5. In the remaining cases a specific operative diagnosis was not recorded.

The pathologic diagnosis (excluding adenofibromatous hyperplasia) made at the time of the original examination in each case in which granulomatous prostatitis was found in a surgically removed prostate gland is given in the accompanying table. In many instances the diagnosis was accompanied by a description of "giant cells, foam cells, lipoid degeneration, plasma cell infiltration," and so forth, and by a statement such as, "granuloma; can't rule out syphilis or tuberculosis."

Cultures of urine were made in 13 cases. In 9 they resulted in no growth. Streptococcus faecalis was found in 2 cases, Aerobacter aerogenes in 1 case and Pseudomonas aeruginosa in 1 case.

Tissues from 4 prostate glands were injected into guinea pigs. They were obtained from prostate glands which were suggestive of, or concerning which the diagnosis was, tuberculosis at the initial pathologic examination, but none of the guinea pigs gave results positive for tuberculosis.

Original Pathologic Diagnosis (Excluding Adenofibromatous Hyperplasia) in the Thirty-Four Cases in Which Granulomatous Prostatitis Was Found in a Surgically Removed Prostate Gland

Diagnosis	Cases
Granulomatous prostatitis	8
Chronic purulent prostatitis	7
Chronic prostatitis	5
Prostatitis	4
Tuberculosis	4
Granuloma (type undetermined)	2
Inflammatory tissue	2
Acute prostatitis	1
Possible lymphosarcoma	1

A long term follow-up investigation was not made, but in the immediate postoperative period sinuses did not form, nor were there other features suggestive of a specific or generalized infection.

The foregoing clinical and laboratory findings revealed little or nothing in favor of a specific etiologic agent (particularly Mycobacterium tuberculosis and Spirochaeta pallida) in the production of the lesion. On the contrary, the clinical features were those of simple prostatic enlargement with obstruction of urinary flow, with physical findings frequently suggestive of carcinoma of the prostate gland.

LITERATURE

A description of a granulomatous lesion of the prostate gland such as that described in this paper could not be found in the literature. The specific granuloma of tuberculous or syphilitic origin is, of course, frequently discussed in the literature on the prostate gland. In order to obtain facts pertinent to this paper, an effort was made to review literature dealing with inflammatory conditions of the prostate gland which might cause lesions similar histologically to granulomatous prostatitis. Also, the literature concerning certain granulomatous lesions in other genitourinary organs was studied, and the contributions which seemed to play a part in the final interpretation of this work are cited in the following pages.

Lesions closely resembling granulomatous prostatitis have been found in the urinary tracts of males and females of all ages.[6] Judging from the number of

6. Braasch, W. F., and Hurley, M. V.: J. Urol. **18**:595, 1927. Thomas, G. J.: S. Clin. North America **3**:1273, 1923.

reported cases, syphilis of the prostate gland is of rare occurrence. If one considers only those cases in which the histologic observations are reported, it is even rarer.[7] The subject of tuberculosis of the prostate gland has had much discussion.[8] The most commonly occurring inflammatory lesion in the prostate gland is the ordinary chronic prostatitis of nonspecific origin. Many studies have been made.[9] Another type of lesion has been described by Heitz-Boyer [10] as "peridiverticulitis" of the prostate gland. Many isolated cases of specific infections of the prostate gland have been reported.[11] That the prostate gland may be affected in the presence of certain generalized bodily diseases which institute a granulomatous type of reaction has been stressed frequently.[12]

Described in the literature are two conditions that may occur in the prostate gland which are not of inflammatory origin but which might be considered as underlying causes of the granulomatous lesion. The first of these is previous prostatic surgical operation.[13] The second is infarction.[14]

COMMENT

In the first portion of this paper a histologic description was given of a lesion found in prostatic tissues surgically removed from 34 patients. The microscopic picture in most of the instances was that of a nonspecific granulomatous reaction closely associated with dilated ducts and acini in the gland. Foreign body giant cells, large mononuclear cells filled with lipoid material and a dense infiltration of lymphocytes and plasma cells characterized the general picture of the reaction. However, in other portions of the same gland there invariably was nongranulomatous chronic inflammation, and the granulomatous type of reaction could be seen to have developed around the ducts where the epithelial lining and wall were being destroyed. This fact suggests that both inflammatory and "foreign body" reactions play roles in bringing about the final picture. Prostatic secretions contain proteins, nucleic acid (as a nucleoprotein) and lipoids (particularly sphingomyelin).[15] That these substances may be changed sufficiently in the presence of infection to be irritating when they escape their epithelial barriers is a likely possibility. This statement is based on the findings of Hirsch,[16] who discussed the relation of the chemical composition of lipids to certain tissue reactions. He found that the

7. Comalli, A., cited by Cabot.[8d] Keyes, E. L.: Am. J. Syph., Gonor. & Ven. Dis. **20**:418, 1936. Thompson, L.: ibid. **4**:323, 1920. Warthin, A. S.: ibid. **2**:425, 1918.

8. (*a*) Barney, J. D.: J. Urol. **10**:81, 1923. (*b*) Bothe, A. E.: ibid. **18**:494, 1927; (*c*) Internat. Clin. **2**:158, 1938. (*d*) Cabot, H.: Modern Urology, Philadelphia, Lea & Febiger, 1936, vol. 1, pp. 86-87; vol. 2, pp. 515-517. (*e*) Moore, R. A.: J. Urol. **37**:372, 1937. (*f*) Strom, G. W., and Thompson, G. J.: ibid. **45**:858, 1941.

9. Gardner, L. W.: Urol. & Cutan. Rev. **44**:278, 1940. Kretschmer, H. L.; Berkey, H. A.; Heckel, N. J., and Ockuly, E. A.: Illinois M. J. **71**:152, 1937. Nichel, A. C.: Proc. Staff Meet., Mayo Clin. **5**:85, 1930.

10. Heitz-Boyer, M.: Mém. Acad. de chir. **66**:440, 1940.

11. Drummond, A. C.: Am. J. Surg. **31**:98, 1936. Lewis, B.; Carroll, G., and Stryker, G. V.: J. A. M. A. **94**:1987, 1930. McGreer, C. F., and McNeil, E.: Proc. Soc. Exper. Biol. & Med. **36**:587, 1937. Riba, L. W., and Perry, E.: J. Urol. **22**:563, 1929. Stuhler, L. G.: Proc. Staff Meet., Mayo Clin. **8**:221, 1933. Teng, C. T., and Forkner, C. E.: Chinese M. J., 1936, supp., p. 394.

12. Boyd, M. L.: J. Urol. **39**:717, 1938. Chopra, S. L.: Indian M. Gaz. **60**:582, 1925. Marchildon, J. W.: Am. J. M. Sc. **140**:74, 1910. Riley, A., and Suby, H. I.: New England J. Med. **217**:616, 1937.

13. Zide, H. A.: Proc. Staff Meet., Mayo Clin. **12**:769, 1937.

14. Abeshouse, B. S.: J. Urol. **30**:97, 1933. Hubly, J. W., and Thompson, G. J.: Proc. Staff Meet., Mayo Clin. **13**:401, 1938.

15. Moore, R. A., and Hanzel, R. F.: Arch. Path. **22**:41, 1936.

16. Hirsch, E. F.: Arch. Path. **31**:516, 1941.

formation of fatty acids from various body lipoids can occur within the body and that these substances may then act as irritants to the tissues.

In other instances previously described the lesion presented a histologic picture suggesting tuberculosis, yet transitions between the nonspecific inflammation associated with the ducts and the pseudotubercles could be found in all cases. This picture was entirely similar to the general picture of granulomatous prostatitis except that the inflammation remained longer within the walls of the ducts and acini, thus giving rise to circumscribed regions suggesting tubercle. The large mononuclear cells, so prominent in the general granulomatous reaction, played important parts in the formation of the pseudotubercles also. Here, the cells became much more elongated, and suggested epithelioid cells. Again, regions of transition could be found to show that these cells and the large mononuclear cells were actually the same. Efforts to prove these cells to be of epithelial origin were not conclusive. The work of Lemon [17] and of Lemon and Feldman [18] furnished precedent for the conception that mononuclear cells can change to "epithelioid" cells. Their work also showed the close histologic similarity between certain foreign body tissue reactions and tuberculosis. In the cases of granulomatous prostatitis efforts to find Mycobacterium tuberculosis were unsuccessful. Furthermore, there was no caseation or even karyorrhexis in the lesion in any of these cases. In addition, the clinical findings in the surgical cases and the results of general examinations in 4 cases in which necropsy was performed failed to reveal evidence of tuberculosis. The guinea pigs that were inoculated remained negative for tuberculosis. From these positive and negative findings, the possibility of tuberculosis was excluded as definitely as possbile.

Syphilitic, mycotic and other specific types of inflammation were well excluded by the histologic, bacteriologic, serologic and clinical observations.

The tissue reactions due to previous prostatic surgical operation and to infarction bear only superficial resemblance to granulomatous prostatitis.

The lesion described by other authors as nonspecific granuloma of the urinary tract is similar to the prostatic lesion in the cases with which this paper is primarily concerned. The literature, however, does not offer a solution of the genesis of even the nonspecific granulomatous lesion and in no instance described was it found in the prostate gland.

The histologic characteristics of chronic prostatitis have been reported, but infrequently, and a description of a group of lesions resembling granulomatous prostatitis could not be found. Certainly the granulomatous prostatitis presented in this paper is related to nonspecific chronic prostatitis, but no single pyogenic organism can be selected as the etiologic agent on the basis of our studies.

It may be of some interest to point out the similarity between the histologic picture of granulomatous prostatitis and that of the disease of the female breast called "plasma cell mastitis." [19]

SUMMARY

A group of inflammatory lesions occurring in surgically removed prostatic tissues presented the general microscopic picture of granuloma and could have been confused histologically with certain specific granulomatous lesions, particularly that of tuberculosis.

17. Lemon, W. S.: Bull. Am. Acad. Tuberc. Physicians **2**:16, 1938; J. Thoracic Surg. **8**:371, 1939.

18. Lemon, W. S., and Feldman, W. H.: Arch. Int. Med. **53**:367, 1934.

19. Cromar, C. D. L., and Dockerty, M. B.: Proc. Staff Meet., Mayo Clin. **16**:775, 1941.

The genesis of the granuloma in question, judging primarily from histologic studies of surgically removed tissue, but aided by clinical, bacteriologic, serologic and postmortem observations, we believe to be as follows: (1) partial obstruction of some of the prostatic ducts either by primary inflammation in them (or inflammation coincident with prostatitis and urethritis) or by mechanical pressure resulting from benign nodular hypertrophy; (2) stasis in smaller ducts and acini, with subsequent intraluminal infection of a chronic, nonspecific type; (3) destruction of the epithelial lining and walls of ducts and acini, with escape of inflammatory products and altered prostatic secretions into the interstitial tissues; (4) production of a diffuse or local chronic inflammatory reaction, plus a "foreign body" type of inflammation, with influx of numerous giant cells, foam cells, plasma cells and lymphocytes; (5) a variation of the fourth process, wherein the chronic inflammation remains longer within the boundaries of duct walls and forms circumscribed nodules of a granulomatous nature, associated with changes in the mononuclear cells to produce pseudotubercles; (6) slow resolution of the lesion in some portions, with complete loss of glandular parenchyma and marked replacement with fibrous tissue.

CHANGES IN THE THYMUS WITH SPECIAL REFERENCE TO MYASTHENIA GRAVIS

OBSERVATIONS IN A SERIES OF SIX THOUSAND AUTOPSIES

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Persistence of the thymic gland in adults and tumors of the thymus have been the subject of many surveys and case reports.[1] It has been postulated that the neoplasms encountered in more than half the cases of myasthenia gravis pseudoparalytica and diagnosed as thymoma constitute a distinct group of tumors, different morphologically from the thymic growths found in other conditions.

The purpose of this study is to present the findings in the thymus in all cases of thymic persistence or tumor that have been encountered in the series of necropsies on adults at the New Haven Hospital since 1918. Among 6,000 autopsies there were found 41 instances of tumor or enlargement of the thymus. Twenty-seven of these concerned children under 16 years of age and will not be considered here. The remaining 14 cases include 3 of cancer (cases 10, 11 and 12), 3 of enlargement of the gland in thyrotoxicosis (7, 8 and 9), 6 of enlargement of the gland encountered incidentally at necropsy (1 to 6), and 2 of noncancerous thymic tumor coincident with myasthenia gravis (13 and 14).

The data concerning these 14 cases and pertinent to the thymus are compiled in the accompanying table. The findings coincident with myasthenia gravis (cases 13 and 14) are detailed in subsequent pages. The patients with myasthenia gravis were the only ones to come to autopsy among the 9 who have died from this disease in the New Haven Hospital since 1921.

REPORT OF CASES

CASE 13.—The patient was a white man of 37 years. The clinical details of his case have been presented elsewhere.[2] This patient, whose past history is unimportant for the purpose here, noted muscular weakness three months after he had undergone tonsillectomy and seventeen years before his death. Difficulty in swallowing was the first symptom, followed by weakness of the arms and the legs. Three years after the onset of symptoms the patient sought admission to the hospital because of prostration. Neurologic examination resulted in a diagnosis of myasthenia gravis. Five years before death the patient started to take 35 mg. of prostigmine bromide daily. With this medication he was able to work as an office manager but found it necessary to be readmitted to the hospital several times. During one of these hospitalizations biopsies were made on the pectoral muscle before and after an injection of a solution of prostigmine methylsulfate. Cholesterinase determinations were made and are reported by Goodman and Gilman in their textbook of pharmacology [3] and by Goodman, Carlson and Gilman.[2] Two days before death the patient returned to the New Haven Hospital with an

From the Laboratory of Pathology, Yale University School of Medicine.

1. (*a*) Bell, E. T.: J. Nerv. & Ment. Dis. **45**:130, 1917. (*b*) Lievre, J. A.: Presse méd. **44**:991, 1936. (*c*) Norris, E. H.: Am. J. Cancer **27**:421, 1936; **30**:308, 1937. (*d*) Obiditsch, R. A.: Virchows Arch. f. path. Anat. **300**:310, 1937. (*e*) Sloan, H. E.: Surgery **13**:154, 1943.

2. A paper on this case was read by L. S. Goodman, R. I. Carlson and Alfred Gilman at the thirtieth annual meeting of the American Society for Pharmacology and Experimental Therapeutics, Inc., in Toronto, April 26-29, 1939 (J. Pharmacol. & Exper. Therap. **66**:15, 1939).

3. Goodman, L., and Gilman, A.: The Pharmacological Basis of Therapeutics, New York, The Macmillan Company, 1941, p. 385.

Data on Fourteen Cases of Thymic Abnormality

Case	Sex	Age	Race	Cause of Death	Weight or Size of Thymus	"Normal" Weight *	Microscopic: Separation Between Cortex and Medulla	Microscopic: Interstitial Tissue	Microscopic: Hassall's Corpuscles	Microscopic: Epithelial Metaplasia	Comment
1	M	19	W	Respiratory infection	28 Gm.	19.3 Gm.	Not distinct	Dense, fibrous	Numerous; young and hyaline	None	Illustrated in fig. 1*B*
2	M	52	W	Respiratory infection	16 × 6 × 1.5 cm.	18.7 Gm.	Visible	Lipoid and fibrous	Few; young and hyaline	Moderate	Acromegaly and polyglandular disease (reported by Cushing †)
3	F	24	W	Respiratory infection	40.5 Gm.	20.5 Gm.	Not distinct	Dense, fibrous	Numerous; hyaline	None	
4	M	40	W	Fat embolism after gunshot	70 Gm.	18.7 Gm.	Not distinct	Lipoid, fibrous	Numerous; hyaline	None	
5	M	35	W	Gunshots	5 × 2 cm.	19.7 Gm.	Distinct	Lipoid, fibrous	Numerous; hyaline	Moderate	
6	F	19	B	Automobile accident	40 Gm.	19 Gm.	Distinct	Lipoid	Numerous; hyaline	None	
7	F	31	B	Thyrotoxic heart disease	32 Gm.	19.7 Gm.	Distinct	Lipoid, fibrous	Numerous; young	Moderate	Thyrotoxicosis (illustrated in fig. 1*A*)
8	F	22	W	Postoperative hemorrhage	26 Gm.	20.5 Gm.	Not distinct	Lipoid, fibrous	Numerous; young and hyaline	Moderate	Thyrotoxicosis
9	F	47	W	Respiratory infection	40 Gm.	18.7 Gm.	Distinct	Lipoid	Numerous; hyaline	Moderate	Thyrotoxicosis
10	M	60	W	Metastases of thymic carcinoma	Cancerous thymoma						
11	M	60	W	Metastases of thymic carcinoma	Cancerous thymoma						
12	F	37	W	Compression of bronchi by thymic carcinoma	Cancerous thymoma						
13	M	37	W	Myasthenia gravis	See fig. 2		Not distinct	Fibrous	Extremely rare	Extreme	Illustrated in fig. 2
14	M	49	W	Myasthenia gravis	See fig. 3		Not distinct	Fibrous	Extremely rare	Extreme	Illustrated in fig. 3

* Blackfan, K. D.: II. Growth and Development of the Child, New York, The Century Company, 1933, p. 345.
† Cushing, H.: The Pituitary Body and Its Disorders, Philadelphia, J. B. Lippincott Company, 1912.

infection of the upper respiratory tract. Respiratory difficulty was enhanced by the paralysis of the accessory respiratory muscles. There were bilateral ptosis, nystagmus on lateral gaze and sluggish reflexes. The clinical diagnosis was pneumonia, myasthenia gravis and malnutrition.

Anatomic Diagnosis.—Generalized muscular atrophy and emaciation; enlargement and focal calcification of the thymus; hemorrhagic and purulent bronchitis and bronchiolitis; focal pneumonia; acute splenic tumor; generalized lymphadenopathy.

Abbreviated Autopsy Record.—The body was that of an emaciated but well developed white man (length 165 cm. and weight 100 lbs. [45.4 Kg.]). Some enlarged lymph nodes were palpable in the axillas and groins. On the skin of the arms and the scapular regions were small red macules. There was generalized muscular atrophy, most marked in the hands and the feet, where the interosseous spaces were deeply depressed. The panniculus adiposus was greatly reduced, and the muscles were pale, flabby and dry. The abdominal situs was normal save for absence of the appendix (scar of appendectomy) and some pericecal adhesions.

There was a firm spherical mass, 6 by 5 by 4.5 cm., anterior to the aortic arch and the base of the heart (fig. 2 *A*). This mass was surrounded by a thick white glistening capsule. The capsule was bound by fibrous adhesions to the anterior pericardial surface; otherwise it was free, not even adherent to the lung that covered the tumor. The latter was largely a calcified mass surrounded by soft friable pink tissue.

The lungs retained their shape and did not collapse when the thorax was opened. They were noncrepitant and dark purple. When cut, they discharged a large quantity of pink frothy fluid, and purulent material welled out of the bronchi. The trachea was red and injected.

The spleen was enlarged and flabby. All lymph nodes were fleshy and enlarged. The remaining organs were grossly normal.

Microscopic Notes.—As regards the thymus, the capsule of the tumor consisted of dense fibrous tissue with scattered foci of round cells. Thick fibrous trabeculae divided the thymic tissue into numerous lobules of various sizes. Some blood vessels in the trabeculae contained organizing thrombi. The thymic lobules consisted of numerous small thymocytes embedded in the meshes of a syncytium of reticulum cells. The latter were numerous in the cortex and not restricted to the medulla as in an infantile thymus. Some of these cells had the typical appearance of epithelial cells and in some places were arranged in "epithelioid" nests. Hassall's corpuscles were extremely scarce, and the few seen were completely hyalinized (figs. 2 *B* and *C*).

In sections taken from different thoracic muscles and from muscles of the extremities there were large areas of lymphocytic infiltration. The muscle fibers in these regions had lost their striations, and the lymphocytes were localized to the interstitial tissue as well as to the zones of necrosis of muscle. Vacuolation as described by Norris[1c] was not observed in these sections (fig. 2 *D*).

In the lymph nodes, the sinuses contained large numbers of polymorphonuclear cells, round cells and mast cells. Their lining cells were swollen. The lymphatic tissue was normal.

The thyroid showed no microscopic sign of thyrotoxicosis, and the follicles were spheric and had no papillary growths. All of them contained colloid and a few vacuoles. No lymphoid tissue was seen.

There was thickening of the pleura by amorphous fibrous tissue, and a few circular fibrotic nodules were seen in one area in the upper lobe of the right lung. Some round cells and epithelioid cells were found, and once a giant cell of the Langhans type. Numerous alveolar lumens contained polymorphonuclear exudate, and similar cells were seen in bronchi.

No myocardial lessions were found, and the myocardial damage as described in myasthenia gravis by Rottino, Poppiti and Rao[4] was not observed.

The microscopic examination of the remaining organs, including the central nervous system and the peripheral nerves, disclosed no abnormality.

Case 14.—A white man of 49 years had been well until seventeen months before his death. At this time he was admitted to the New Haven Hospital because of difficulty in swallowing and progressive weakness. Weakness of both the arms and the legs and occasional double vision, noted after some exertion, and then increasing considerably, had been his first symptoms. The neurologic examination disclosed no abnormality. Tests with prostigmine methylsulfate gave results as follows: Tonometer readings before the prostigmine compound was given were: right, 75; left 60. Those after the prostigmine compound was given were: right 85; left 78. Biopsy of the deltoid muscle showed no abnormality. The patient was discharged on a dosage of 45 mg. of prostigmine bromide daily. He felt well for a few months; then his weakness recurred in spite of an increase in the dose of prostigmine bromide. For a short period he felt subjectively better without prostigmine but the tonometer readings were: right 60; left 45.

4. Rottino, A.; Poppiti, R., and Rao, J.: Arch. Path. **34**:557, 1942.

† Cushing, H.: The Pituitary Body and Its Disorders, Philadelphia, J. B. Lippincott Company, 1912.

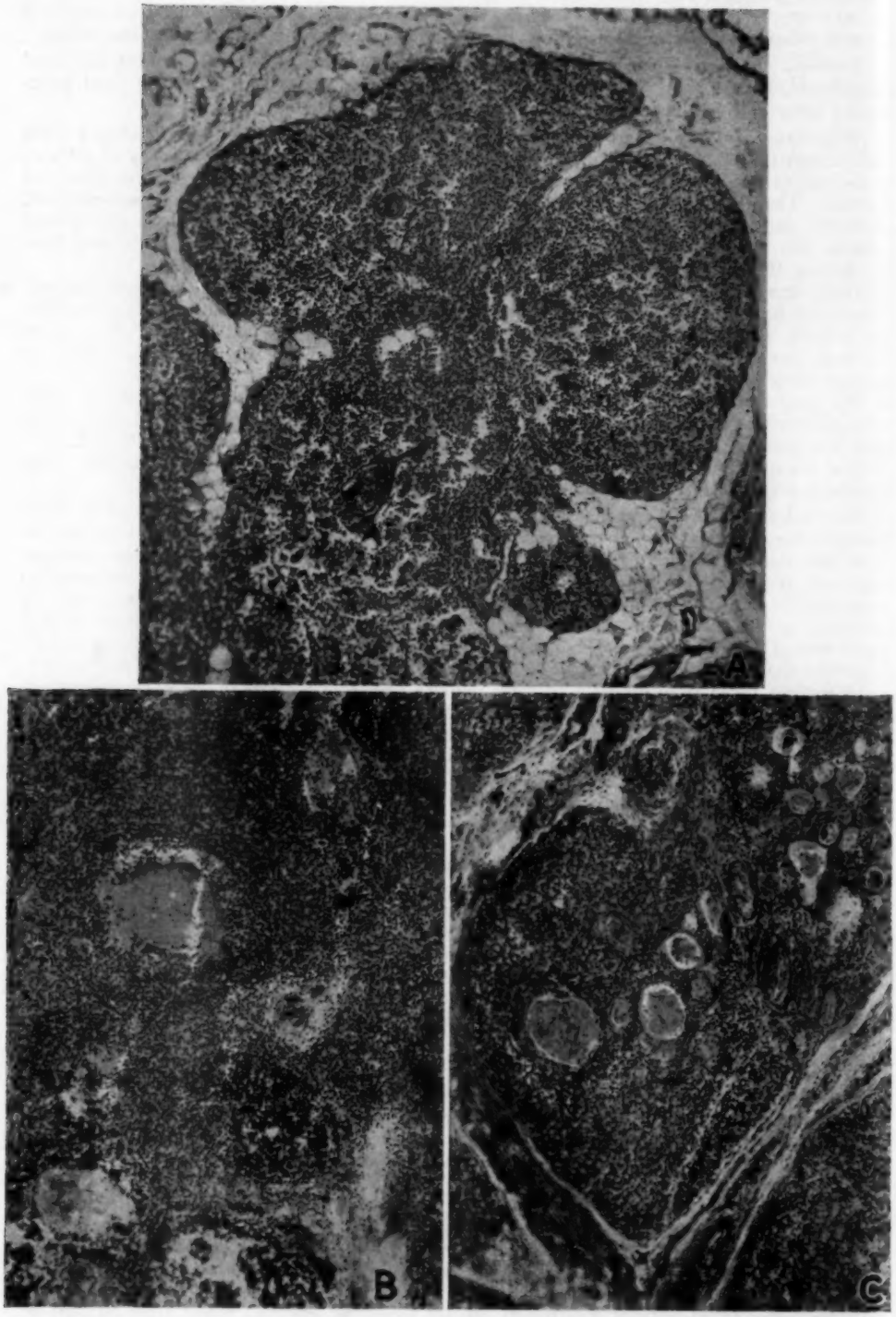

Figure 1

(See legend on opposite page)

Later, without medication, the patient's muscular strength became: right 101; left 120. This remission lasted four months, after which the patient was again hospitalized because of difficulty in swallowing and progressive weakness. Prostigmine at this time was of little avail. Thymectomy was discussed, but the patient improved with 40 mg. of prostigmine bromide daily, and refused exploratory thoracotomy. His strength as tested by tonometer was: right 120; left 90. He was able to swallow normally. After omission of the drug the symptoms recurred. The patient, still refusing operation, was discharged on a dosage of five times 60 mg. of prostigmine bromide in twenty-four hours. One month later he was readmitted with grave weakness of the respiratory muscles, unable to move or speak. Intravenous injection of a solution of prostigmine methylsulfate helped only for a few minutes (five days before his death), and then he had to be placed in a respirator. He became incontinent four days before death and succumbed after repeated spells of cyanosis.

The clinical diagnosis was myasthenia gravis. The laboratory data were negative.

Anatomic Diagnosis.—Epithelial thymoma; decubiti; focal pneumonia (bilateral); congestion of viscera; hydrothorax (left); multiple cerebral petechiae; petechiae in the pancreas; hematoma of the scalp; subsidiary conditions (fibrosis of testicles; hyperplasia of the islets of Langerhans).

Abbreviated Autopsy Record.—The body weight was 190 pounds (86 Kg.); the length, 180 cm. Decubital ulcers in the region of both buttocks were the only findings on external examination. The panniculus adiposus was fairly well preserved. The abdominal organs had the usual anatomic relationships. There were fibrous pleural adhesions in both apical regions, and the consistency of the lungs was generally increased. There was water-like transudate in the left pleural cavity.

Anterior to the arch of the aorta and in the region of the thymus there was a mass measuring 8 by 5 by 2 cm. Its consistency was firm in some places and elastic and somewhat fluctuant in others. Its color was pale yellow. On the right side it was adherent to the mediastinal pleura, and posteriorly, to the superior vena cava and to the anterior surface of the pericardium (fig. 3). It was well limited by a thin fibrous capsule. Sections of the mass revealed soft friable tissue divided by fibrous trabeculae into numerous lobules measuring 0.5 to 2 cm. in diameter. Some areas were red and spongy, owing to hemorrhage, and there were some blood-filled cysts, each the size of a lobule.

The lungs were firm in consistency and purple. Large amounts of frothy pink fluid were discharged from the cut surface, and there were numerous foci of pneumonia.

Sections of the brain revealed numerous petechial hemorrhages scattered throughout the hemispheres and the basal ganglions.

The remaining organs were grossly normal.

Microscopic Notes.—The tumor of the thymus was encapsulated by firm dense fibrous tissue. Fibrous septums extending into the parenchyma and dividing it into small lobules contained lymphatics and blood vessels and in some places nests of small lymphocytes. The lobules consisted largely of reticulum cells as seen in the medulla of the infantile thymus. However, these cells formed not only a syncytium but a real epithelial tissue surrounding scattered thymocytes (fig. 3 *B* and *C*). There was no visible distinction between the cortex and the medulla of the thymus. Hassall's corpuscles were scarce. As described grossly, there were numerous cysts of varying size, lined by epithelial cells like those described in foregoing paragraphs and filled with blood or with pink amorphous material.

The muscles revealed no lymphorrhagia as described in the preceding case. (Norris [1c] reported that this occurs in only two thirds of the cases.) In some places, a loss of striation was noted, and the muscle fibers had pyknotic nuclei.

The lungs showed marked edema, congestion and extensive focal pneumonia.

EXPLANATION OF FIGURE 1

A, delayed involution of the thymus in thyrotoxicosis (case 7 in table); × 75. Note the distinct separation of cortex and medulla, the moderate epithelial metaplasia, the presence of Hassall's corpuscles, and the fibrous and lipoid stroma.

B, persistent thymus (case 1); × 75. It was observed incidentally at autopsy. Note that the separation of cortex and medulla is indistinct, that there are numerous young and also hyalinized corpuscles of Hassall and that there is no epithelial metaplasia.

C, infantile thymus; × 75. This thymus was removed from an infant of 6 weeks. Note the fairly distinct separation of cortex and medulla, the large number of young Hassall's corpuscles, the presence of an epithelial syncytium and the scarce stroma.

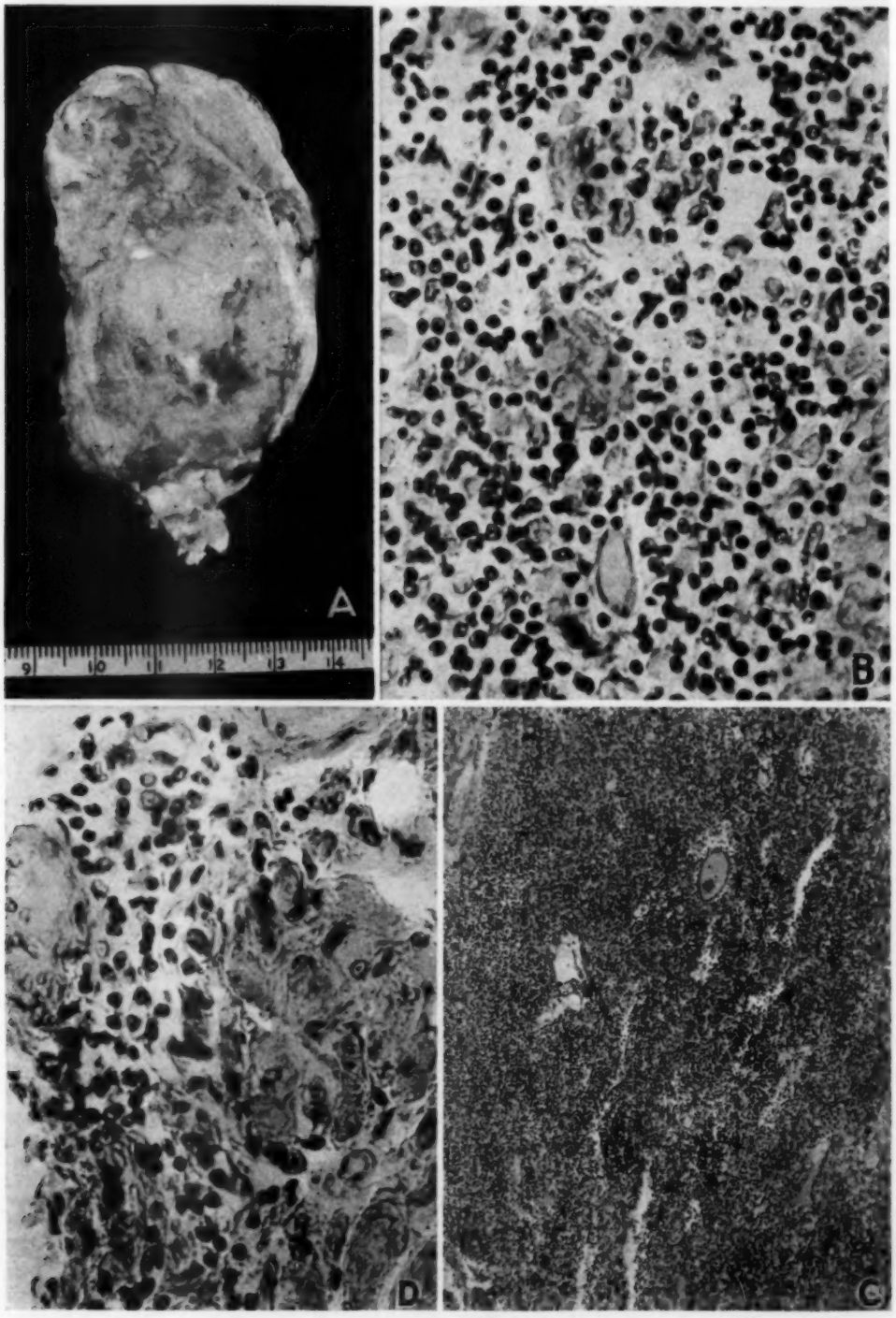

Figure 2

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Sections of the brain revealed numerous foci of perivascular microglial proliferation and perivascular hemorrhage. In this connection it is interesting to remember that this patient received an intravenous injection of prostigmine methylsulfate five days before his death. Examination of the peripheral nerves showed no abnormality.[5] The slides of other organs were free of lesions save for petechiae in the pancreas and fibrosis of the testicles.

LITERATURE

A review of the literature dealing with the incidence of thymic tumors in patients with myasthenia gravis, the experimental and clinical investigations on this subject and the histology of the thymus follows:

Incidence of Thymic Tumors in Patients with Myasthenia Gravis.—In 1941 Poer [6] summarized numerous previous case reports and reviews. Thymic abnormality in the form of persistence of the thymus, enlargement of the thymus or thymic tumor was reported by him to have been noted in 71, or 55.04 per cent, of 129 autopsies and operations on patients with myasthenia gravis. Barnes [7] added another case of myasthenia gravis and thymic tumor.

Experimental Investigations.—In 1903 Basch [8] succeeded in reducing the galvanic irritability of the muscles of thymectomized animals by injections of thymic extract. Similar effects, however, were obtained with adrenal extract. Thymic glands implanted into the omenta of such animals failed to have any influence, but thymic glands grafted into the subcutaneous tissue caused muscular twitching and paralysis ending in death. Hart and Nordmann [9] were able to reproduce these results. Mice given injections of a saline extract of the thymus in case 13 failed to show any changes.

Typical myasthenia gravis, curable by prostigmine, is claimed by Adler [10] to have been produced by repeated implanting of infantile thymus into dogs.

Clinical Investigation.—Harvey, Lilienthal and Talbot [11] studied neuromuscular transmission in patients before and after removal of the thymus. They were impressed by "the similarities between partial curarization and myasthenia gravis, suggesting that the neuromuscular effect in myasthenia gravis might result from the action of some circulating substance," and they referred to the thymus as a "likely source of this hypothetical agent."

Further observations in man after therapeutic thymectomy are of interest, and references may be found in the papers of Poer,[6] Sloan [1e] and others.

Histology of the Thymus in Myasthenia Gravis.—Extensive analysis of the literature led Bell [1a] to conclude that the thymic tumors in patients with myas-

5. The examinations of the central nervous system and of the peripheral nerves reported in this paper were done by Dr. H. M. Zimmerman.

6. Poer, D. H.: Am. J. Surg. **115**:586, 1942.

7. Barnes, G.: Arch. Path. **32**:659, 1941.

8. Basch, K.: Wien. klin. Wchnschr. **16**:893, 1903; **68**:668, 1908.

9. Hart, C., and Nordmann, O.: Berl. klin. Wchnschr. **47**:814, 1910.

10. Adler, H.: Arch. f. klin. Chir. **189**:529, 1937.

11. Harvey, A. M.; Lilienthal, J. L., and Talbot, S. A.: J. Clin. Investigation **21**:579, 1942.

EXPLANATION OF FIGURE 2

A, tumor of the thymus with large central area of calcification (case 13). This tumor was associated with myasthenia gravis.

B, section from the tumor shown in *A*; × 435. Note the epithelial cells and scarce lymphocytes.

C, section showing absence of Hassall's corpuscles; × 75.

D, lymphorrhagia in muscle and muscular atrophy in the same case; × 435.

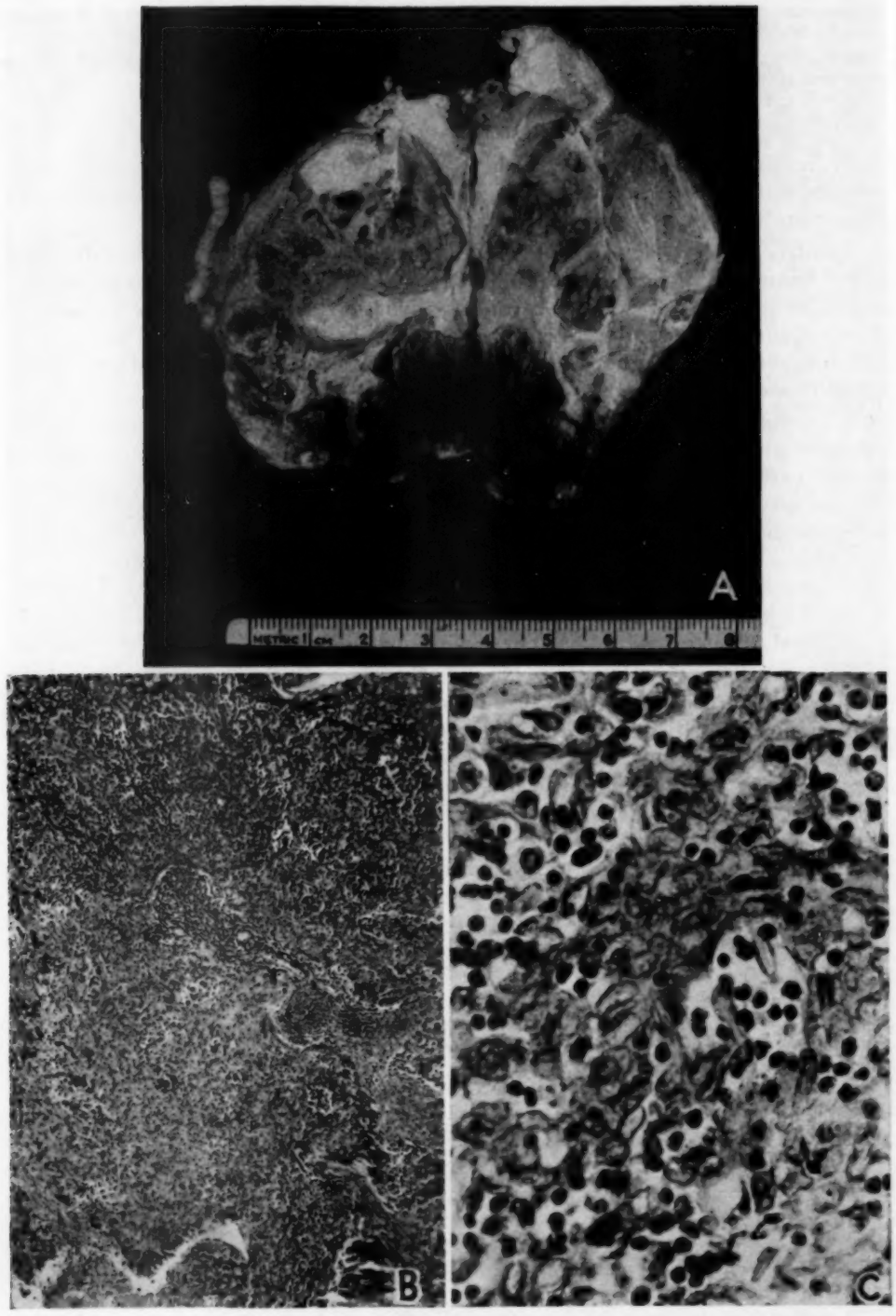

Figure 3

(See legend on opposite page)

thenia gravis "form a distinct group," and Lievre [1b] expressed the belief that these tumors are constant in type. His opinion follows (translation):

. . . It [the tumor] is a mass surrounded by a perfect capsule of connective tissue and sometimes divided by connective tissue trabeculae containing vessels and a variable quantity of stroma. The parenchyma consists of two types of cells: small lymphoid cells arranged without definite pattern—sometimes, however, arranged in a concentric group—and epithelioid cells with vesiculated nuclei, often forming palisades in which the vessels are seen. In places there is syncytial arrangement. Hassall's corpuscles are infrequent. This structure resembles the embryonic thymus which passes from an epithelial structure to a lymphoid one.

In 1936 Norris [1c] divided thymic tumors found in patients with myasthenia gravis into two groups: (1) noncancerous tumors, which represent a condition of extreme epithelial metaplasia, and (2) enlarged or persisting thymic glands, representing a condition of moderate epithelial hyperplasia. Cancerous thymic tumors in patients with myasthenia gravis, such as those reported by Poer,[6] have been observed as a third group.

Obiditsch [1d] described in cases of myasthenia gravis "a predominance of lymphoid elements without any cancerous changes [in the thymus]," whereas thymic tumors unacompanied by myasthenia gravis "gradually become cancerous and show predominance of epithelioid elements." Sloan [1e] similarly noted a large number of lymphoid elements in thymic glands removed at operation from patients with myasthenia gravis.

COMMENT

Two thymic tumors observed in patients with myasthenia gravis pseudoparalytica differed histologically from the 12 pathologic thymus glands encountered in 6,000 necropsies performed since 1918 (cases 13 and 14).

The thymic tumors accompanied by metastases were diagnosed as cancerous thymoma (cases 10, 11 and 12).

The enlarged thymuses from patients with thyrotoxicosis contained a large number of thymocytes and resembled the thymus of youth. They regularly showed moderate epithelial metaplasia in the medulla and around the corpuscles of Hassall (cases 7, 8 and 9).

The enlarged thymic glands found incidentally at necropsies also showed varying degrees of epithelial metaplasia, similarly placed, and numerous Hassall's corpuscles (cases 1, 2, 3, 4, 5 and 6).

The thymic tumors found in association with myasthenia gravis presented extreme epithelial metaplasia, reduction of the number of small thymocytes and practical absence of Hassall's corpuscles.

It may be by chance that the 2 patients who came to autopsy, among the 9 who have died from myasthenia gravis at this hospital since 1921, had thymic tumors. The fact that persistence of the thymus and myasthenia gravis both are rare makes their coexistence more significant. The argument that such tumors are also found in the absence of myasthenia gravis does not apply to this large series of autopsies since the thymic tumors in such cases were morphologically different from those encountered in cases of myasthenia gravis.

EXPLANATION OF FIGURE 3

A, thymic tumor with cysts and hemorrhages (case 14). This tumor was found in a patient with myasthenia gravis. *B*, section of tumor shown in *A*; × 75. Note the scarcity of thymocytes, the extreme epithelial metaplasia and the absence of Hassall's corpuscles.

C, section of the same tumor showing strands of epithelial cells and scattered thymocytes; × 435.

In reference to table 1, it can be said that in this series the first 9 cases, comprising the thymic changes observed incidentally at autopsy, and the cases of thyrotoxicosis may be considered as instances of persistence of the gland resulting from inhibition of its involution. In all of them the corpuscles of Hassall were fairly numerous.

Cases of incidentally observed thymic persistence rarely show epithelial metaplasia, which is constantly found in thyrotoxicosis.

The thymic enlargements in 2 patients with myasthenia gravis in this series were both noncancerous tumors, characterized by extreme epithelial metaplasia and by quasicomplete absence of the corpuscles of Hassall.

The 3 thymic cancers were diagnosed as carcinoma.

SUMMARY

In the course of a series of 6,000 autopsies 14 instances of thymic enlargement in adults were encountered. There were 3 cases of carcinoma and 3 of persistence of the thymic gland in association with thyrotoxicosis. In 6 instances the thymic abnormality was an incidental finding at autopsy. There were 2 cases of noncancerous thymic tumor coincident with myasthenia gravis.

Thymic epithelial metaplasia was moderate in all patients with thyrotoxicosis but was rare in those in whom enlargement of the thymus was observed incidentally post mortem.

In contrast to this, epithelial metaplasia was an outstanding feature and was accompanied by extreme scarcity of the corpuscles of Hassall in the 2 thymic tumors associated with myasthenia gravis described in the foregoing pages. This is in accordance with the conclusions of Bell,[1a] Lievre [1b] and Norris [1c] that the thymic tumors found in patients with myasthenia gravis are of a distinct type, characterized by epithelial metaplasia; it is in contradiction to the more recent opinion of Obiditsch [1d] and Sloan, [1e] who stress the predominance of lymphoid tissue in thymic tumors of patients with myasthenia gravis.

EXPERIMENTAL STUDIES IN CARDIOVASCULAR PATHOLOGY

IX. REACTIONS IN THE BLOOD AND ORGANS OF DOGS ON INTRAVENOUS INJECTION OF A SOLUTION OF GLYCOGEN

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Previous investigations [1a] dealt with the reactions in the blood and organs of dogs, rabbits and rats following single and repeated injections of various non-physiologic and relatively stable macromolecular substances (polyvinyl alcohol, methyl cellulose, acacia, pectin, gelatin, ovalbumin). It was found that when macromolecular carbohydrates were injected they elicited a macromolecular hematic syndrome in addition to storage phenomena and atheromatosis in small and large vessels and that when foreign-proteinic macromolecules were introduced in excessive amounts and over long periods they produced arteriosclerotic lesions.

It was therefore of interest to extend these studies to the single and the repeated intravenous injection of appreciable amounts of physiologic macromolecular substances, such as homologous plasma proteins and glycogen, which are susceptible to action of the normal degradation mechanisms available to the organism. Animal starch was chosen as the macromolecular agent to be tested in the experiments reported here.

EXPERIMENTAL PROCEDURE

Two adult female dogs weighing 3.0 Kg. and 4.0 Kg., respectively, at the start of the experiment were given an intravenous injection of 10 cc. of a 5 per cent solution of glycogen (Pfanstiehl) in isotonic solution of sodium chloride. Glycogen designated as chemically pure was used for the study of the acute hematic reactions following a single injection of 10 cc. of a 5 per cent solution of the polysaccharide, while in the subsequent administrations the technical variety was used. The solution, which had a viscosity of 1.45 at 19.5 C., was always freshly prepared, filtered through filter paper and injected immediately. The repeated injections were given at the rate of five a week and consisted of fourteen doses of 20 cc., six doses of 45 cc. and one dose of 60 cc. of a 5 per cent solution. A total of 38 Gm. of glycogen was injected into each dog within thirty days. One of the dogs died several hours after the last injection, whereas the other was killed by intravenous injection of a 4 per cent formaldehyde solution twenty-four hours later.

GENERAL OBSERVATIONS

The appetite of both dogs was diminished during part of the experiment. One animal decreased in weight from 4 Kg. at the start to 2.5 Kg. at the end, while the other showed only a transitory minor reduction in weight during the middle part of the experimental period, followed by recovery to the original weight at its end.

The urine was negative for acetone during the entire course of the experiment. Sugar was found only during the last one to two weeks, in quantities of 1+, while albumin was either absent or present as a faint trace. The sediment was without any significant findings.

ACUTE HEMATIC REACTIONS FOLLOWING A SINGLE INJECTION OF GLYCOGEN SOLUTION

In the study of the acute hematic reactions following the injection of 10 cc. of a 5 per cent solution of glycogen (chemically pure) it was noticed that the blood turned black-red

From the Warner Institute for Therapeutic Research.

1. (*a*) Hueper, W. C.: Arch. Path. **33**:267, 1942. (*b*) Hueper, W. C., and Ichniowski, C. T.: Unpublished data.

and that the erythrocytes were conglutinated to form small clumps of cells. The type and number of tests made and the reactions obtained are illustrated in table 1, which gives the data on dog 1317 as a representative example.

The viscosity of the plasma was determined with the Hess viscosimeter. The average size of erythrocytes was obtained by the use of Haden's halometer. The capillary method was employed for the determination of the coagulation time, while Wintrobe tubes were used for the sedimentation test.

Blood smears were stained with Best's lithium carmine stain for glycogen. The leukocytes showed a distinct red color before and after the injection of the glycogen solution.

TABLE 1.—*Acute Hematic Reactions After a Single Injection of Glycogen Solution*

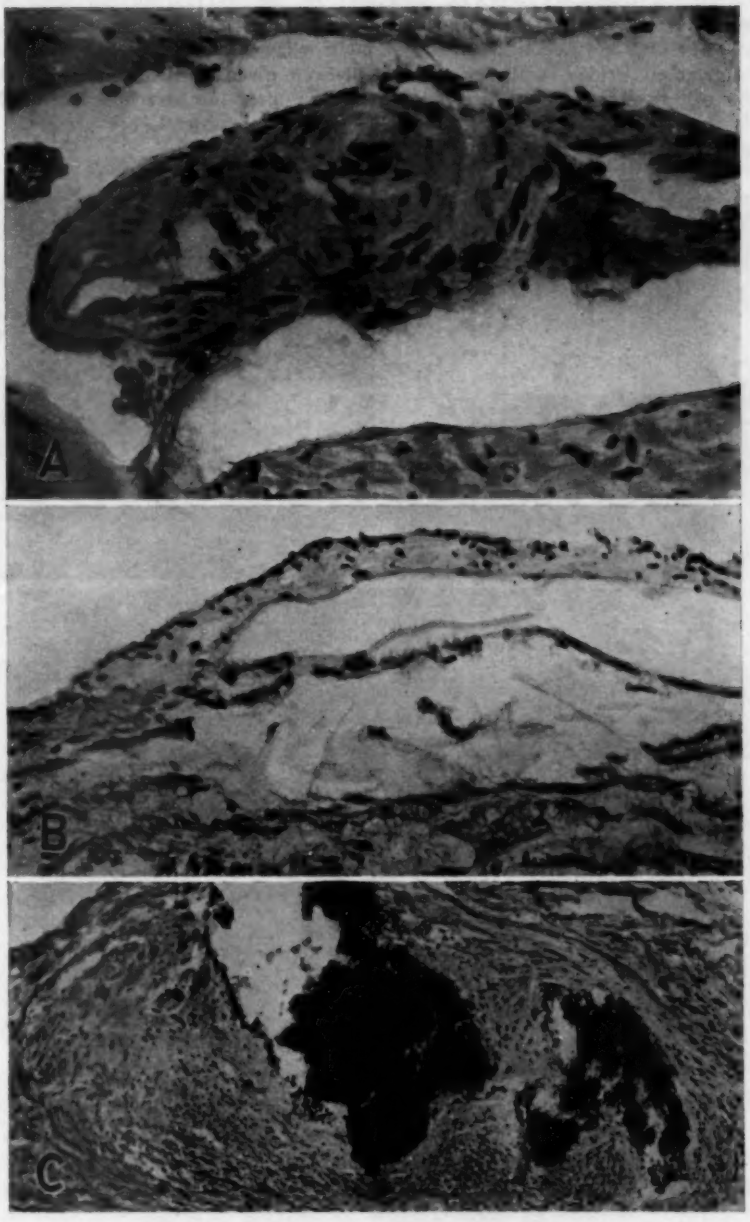
Time	0	5 Min.	15 Min.	45 Min.	2 Hr.	4 Hr.	6 Hr.	24 Hr.
Erythrocytes, millions	6.20	5.25	5.10	5.60	5.85	6.20	6.20	4.05
Hemoglobin, Gm. per 100 cc.	10.3	10.3	10.55	12.95	12.95	11.95	17.70	8.85
Leukocytes, thousands	5.8	6.1	7.1	7	5.5	34.6	47.7	32
Polymorphonuclears, per cent	67	87	72	78	77	82	85	95
Juveniles, per cent	5	6	8	3	7	11	14	2
Eosinophils, per cent	7	7	6	4	9	3	1	0
Basophils, per cent	0	0	0	0	0	0	0	0
Mononuclears, per cent	13	3	8	3	3	1	0	0
Lymphocytes, per cent	8	2	6	12	5	3	0	0
Irritation forms, per cent	0	1	0	0	0	0	0	0
Normablasts, per cent	0	0	0	2	2	1	2	2
Megaloblasts, per cent	0	0	0	5	0	0	0	0
Erythrocytic size, microns	7.60	6.95	7.05	7.4	7.25	6.50	7.00	6.70
Coagulation time, min.	6	8.25	14	13	9.25	11	12	9.25
Sedimentation rate, mm.	9	11	10	26	31	33	44	57
Volume of packed cells, mm.	36	35	36	41	44	40.5	39	33
Plasma viscosity	2.0	2.0	2.0	2.0	2.1	2.0	1.85	1.95
Temperature, C.	19.0	19	19	19	19	19	19	21

TABLE 2.—*Hematic Reactions Following Repeated Injections of Glycogen Solution*

Time in days	2	3	4	6	10	13	17	20	24	27	30
Erythrocytes, millions	5.35	4.85	4.20	4.75	4.10	4.95	3.65	4.40	4.42	4.10	3.95
Hemoglobin, Gm. per 100 cc.	8.90	8.19	8.2	8.2	7.5	7.9	7.2	7.7	9.4	8.9	....
Leukocytes, thousands	10.6	7.7	10.2	32.0	18.0	48.0	19.8	24.2	19.2	18.4	23.6
Polymorphonuclears, per cent	..	83	..	55	60	50	79	67	76	73	..
Juveniles, per cent	..	9	..	8	11	14	1	2	3	3	..
Eosinophils, per cent	..	0	..	0	1	0	0	5	5	7	..
Mononuclears, per cent	..	6	..	20	21	24	11	15	13	6	..
Lymphocytes, per cent	..	3	..	16	7	9	7	11	3	9	..
Irritation forms, per cent	..	0	..	1	0	3	2	0	0	2	..
Normoblasts, per cent	..	0	0	1	0	0	0	0	0	2	..
Erythrocytic size, microns	6.6	6.9	6.95	7.4	7.5	7.6	7.3	..	..	..	..
Coagulation time, min.	9	..	12	10	17	17	20	18	10	12	13
Sedimentation rate, mm.	55	58	63	63	65	67	63	60	8	59	61
Volume of packed cells, mm.	33	33	29	29	27	29	26	28	31	31	28
Plasma viscosity	2.0	1.95	1.95	2.05	2.05	2.25	1.9	2.05	2.05	2.5	2.25
Temperature, C.	20	19	20	23	21	19	23	23	19	17	23

There was no intensification of the color after the injection. The presence of dye precipitates prevented reliable interpretation as to the presence of glycogen in the plasma.

The recorded evidence shows that following the intravenous introduction of a colloidal solution of glycogen there developed definite transitory leukopenia followed by considerable leukocytosis. During the leukopenic phase all types of leukocytes became significantly reduced in number, while the neutrophilic leukocytes predominated during the subsequent leukocytotic stage. The simultaneous increase of juvenile forms of erythrocytes and leukocytes indicates that the latter reaction is associated with an irritation of the bone marrow. Conforming with the colloidoclastic character of these responses there occurred progressive acceleration of erythrocytic sedimentation. The only other significant hematic reaction noted was the primary moderate and transient as well as the severe and delayed decreases in the number of circulating erythrocytes. The hemoglobin values fluctuated essentially parallel with the erythrocytic values and those of the volume of the packed cells. Coagulation time, plasma viscosity and average erythrocyte size remained within normal limits.



EXPLANATION OF FIGURE 1

A, myocardial artery with focal hyalinization and swelling of the wall and clumping of round hyperchromatic nuclei.

B, edematous swelling of the intima and media in the aortic bulb. Ill defined and star-shaped cells with round hyperchromatic nuclei are embedded in the edematous matrix of the subendothelial zone.

C, large fibrous and hyaline area with central calcification in the outer third of the media of the ascending aorta.

OBSERVATIONS FOLLOWING REPEATED INJECTIONS OF GLYCOGEN SOLUTION

The subsequent course of repeated injections of the solution of glycogen was started immediately after the twenty-four hour period of observation of the acute hematic reactions. Blood tests were made twice a week, i. e., on Monday, when two days without injections had passed, and on Friday, when injections had been given on four successive days. Thus the hematic record of this experiment starts two days after the first injection of the solution and twenty-four hours after the second one. The observations made are presented in table 2 giving the data on dog 1317.

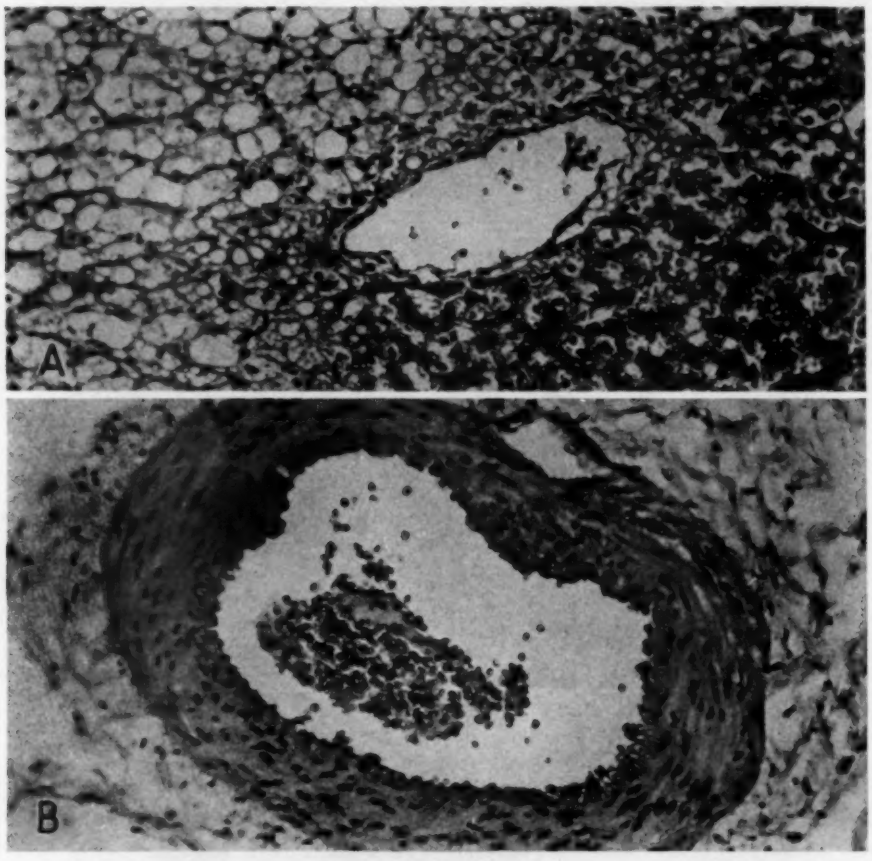


Fig. 2.—*A*, subcapsularly located foam cellular area of liver cells; *B*, renal artery with two small cushion-like cellular thickenings in a locally hyalinized intima.

Blood.—The number of erythrocytes, the amount of hemoglobin and the volume of packed blood cells showed a definite downward trend in both dogs. The leukocytes remained in general at an elevated level, at times reaching 48,000 in both dogs. The clotting time was lengthened during the latter part of the experimental period when larger amounts of glycogen were administered. The sedimentation rate continued to be definitely increased. Plasma viscosity and erythrocyte size remained within normal limits.

Organs.—The observations made at autopsy did not differ appreciably from those made on normal dogs. The livers were rather large, deep brown-red and firm and had a reflecting cut surface. A navy bean-sized yellow-white ill defined area was found in one liver. In both dogs the renal cortex exhibited a few white pinhead-sized spots. All other organs (brain, thyroid gland, lung, heart, aorta, stomach, intestine, skeletal muscle, bone marrow, pancreas, spleen, adrenal glands, uterus, bladder) were normal.

The histologic study of sections of these organs stained with hematoxylin-eosin showed the following changes:

Heart: Several myocardial arterioles, particularly those situated in the papillary muscle of the left ventricle, revealed thickened walls, the increase being partly caused by localized hyaline intimal thickenings protruding into and narrowing the lumen and partly by local swelling and homogenization of the media with accumulation of round, irregularly grouped nuclei (fig. 1 *A*). The myocardium was normal.

Aorta: A minor edematous intimal swelling of crescent shape containing some star-shaped cells was present in the aortic bulb of one dog (fig. 1 *B*). The aorta of the second dog showed in one segment focal hyalinization in the outer part of the media associated with a larger calcified area embedded in hyaline matter (fig. 1 *C*). Numerous other segments of the ascending, thoracic and abdominal aorta as well as of the arch and other large elastic arteries (iliac and carotid arteries) were normal.

Lung: Some capillaries were distended by clumps of conglomerated erythrocytes.

Liver: The hepatic cells were swollen and had a foamy cytoplasm or a cytoplasm with small vacuoles. The sinusoids were congested and contained an increased number of leukocytes and Kupffer cells. These conditions were most pronounced in the pericentral area. One liver contained a subcapsularly located navy bean-sized ill defined area composed of huge foam cells (fig. 2 *A*).

Kidney: Several small mononuclear foci were present in the cortex. The renal artery of one dog exhibited a small hyaline intimal thickening which at its base was ill defined toward the media (fig. 2 *B*). The internal elastic membrane was distinct and swollen in the entire circumference except for the area beneath the intimal thickening, where it was fragmented.

Bone Marrow: A dense myeloid tissue filled the marrow space of the sternum.

All other organs (brain, thyroid and parathyroid glands, lymph nodes, spleen, stomach, intestine, adrenal glands, bladder, skeletal muscle, pancreas) were normal.

Sections from small pieces of several organs (liver, kidney, skeletal muscle, thyroid gland, heart, brain, aorta) which had been fixed in absolute alcohol were stained with Best's carmine method for glycogen. Small amounts of red-stained granules were demonstrated in the brain cells, liver cells, skeletal muscle and heart muscle. The other organs were negative for glycogen. The glycogen granules present in the liver cells were arranged in an irregular network surrounding the numerous small vacuoles contained in the cytoplasm. In the myocardium the glycogen granules exhibited a cross-striated arrangement.

Frozen sections of liver tissue fixed in solution of formaldehyde and stained with sudan III revealed the presence of scanty or moderate amounts of red-stained droplets in the pericentral liver cells of the 2 dogs.

COMMENT

The hematic reactions elicited when a colloidal solution of glycogen was injected intravenously into dogs are similar to those following the intravenous introduction of other macromolecular colloidal agents and are characteristic of the macromolecular hematic syndrome. These observations in general confirm those concerning primary transitory leukopenia and secondary leukocytosis following the intravenous injections of glycogen previously made in rabbits and dogs by others.[2] While Staub and co-workers noted in rabbits during the leukopenic phase a disappearance of the myeloid leukocytic elements and in severe reactions a reduction in the absolute number of lymphocytes, the present studies on dogs did not reveal such selective immobilization of certain types of leukocytes during the leukopenic phase but showed that all types were approximately equally involved in this reaction. Staub and Bucher[2c] once contended that the myeloid leukocytes are arrested temporarily in the pulmonary capillaries because these cells being normally larger than the lymphocytes adsorb or phagocytose the colloidal carbohydrates. They become thereby either more voluminous aggregates or they become less pliable and therefore less ready to pass through the pulmonary capillaries, as the phagocytosed glycogen may saponify the cytoplasmic matter. It was demonstrated

2. (*a*) Staub, H.; Mezey, K., and Golandas, G.: Klin. Wchnschr. **17**:1501, 1938. (*b*) Staub, H., and Golandas, G.: Schweiz. med. Wchnschr. **68**:969, 1938. (*c*) Staub, H., and Bucher, K.: Klin. Wchnschr. **17**:1555, 1938. (*d*) Bucher, K.: Arch. f. exper. Path. u. Pharmakol. **191**:587, 1939. (e) Bucher, K., and Staub, H.: ibid. **194**:506, 1940.

in previous experiments with other macromolecular polymers of carbohydrate nature (polyvinyl alcohol, methyl cellulose, pectin, acacia), which gave similar leukopenic reactions, that the carbohydrates then used were not phagocytosed by the leukocytes, while the present studies showed that the leukocytes of the circulating blood were not loaded with glycogen to an increased degree after repeated injections of considerable amounts of glycogen. The observations made indicate at the same time that the colloidal carbohydrates are not adsorbed to a demonstrable and appreciable degree to the surface of the myeloid leukocytes, thereby increasing their caliber. It appears to be more probable that the colloidal plasmatic disturbances brought about by the introduction of glycogen or any other high polymer carbohydrate and causing a tendency of the erythrocytes to conglutinate to clumps is involved in the arrest of the red and white cells in the pulmonary capillaries.

The hematic reactions following repeated injections of glycogen solution also conform to the symptoms seen in the macromolecular hematic syndrome. There were increased sedimentation rate, leukocytosis or hyperleukocytosis, reduction in the number of erythrocytes and lengthening of the clotting time. These responses to transitory hyperglycogenemia are similar to those in glycogen storage disease, which is characterized by a marked increase of the glycogen and cholesterol content of the blood and which is associated with anemia, accelerated erythrocytic sedimentation and hemorrhagic diathesis.[3] It may be mentioned in this connection that the urine of persons with glycogenosis may contain sugar (Unshelm [3b]), a reaction observed also in the 2 dogs toward the end of the experiment when considerable amounts of glycogen were injected daily.

Although the present studies did not include any experiments on the severity and the duration of the hematic reactions with regard to the amount of glycogen injected and the type of glycogen used, it may be mentioned that Bucher [2d] found that the severity and the duration of the leukopenia depended directly on the quantity of glycogen introduced. This investigator noted, moreover, that glycogen prepared from rabbit's liver was in hematic respects less effective than glycogen obtained from the livers of clams and hogs. Bucher suspected that the known marked variations in molecular size of glycogen depending mainly on the method employed in its isolation may be responsible for such differences.

In the absence of any disturbance of the glycogen metabolism it was not to be expected that the organs of the dogs would show pathologic changes such as those found in glycogen storage disease even under the influence of prolonged administration of glycogen. The livers fixed in absolute alcohol immediately after death contained only minor amounts of glycogen but showed definite hydropic swelling and minor to moderate fatty infiltration of the liver cells, indicating a certain disturbance in the vital activities of these cellular elements.

While no reports are available on the occurrence of arteriosclerotic or atheromatous vascular lesions in persons with glycogen storage disease possibly caused by the plasmatic colloidal disturbances accompanying the existing hyperglycogenemia and hypercholesteremia,[4] the aorta and the myocardial and renal arteries of the 2 dogs studied exhibited intimal and medial lesions which may have a causal relation

3. (*a*) von Gierke, E.: Beitr. z. path. Anat. u. z. allg. Path. **82**:497, 1929. (*b*) Unshelm, E.: Jahrb. f. Kinderh. **137**:257, 1932. (*c*) van Creveld, S.: Klin. Wchnschr. **12**:529, 1933. (*d*) Biedermann, G., and Hertz, W.: Ztschr. f. Kinderh. **56**:170, 1934. (*e*) Krakower, C.: J. Pediat. **9**:728, 1936. (*f*) Beumer: Klin. Wchnschr. **16**:654, 1937. (*g*) Wagner, R.: Ergebn. d. inn. Med. u. Kinderh. **53**:586, 1938.

4. Antopol, W.; Heilbrunn, J., and Tuchman, L.: Am. J. M. Sc. **188**:354, 1934. Siegmund, H.: Klin. Wchnschr. **18**:624, 1939. Antopol, W.; Boas, E. P.; Levison, W., and Tuchman, L. R.: Am. Heart J. **20**:546, 1940.

to the colloidal plasmatic disorder elicited by the repeated injections of glycogen solution. The veil-like edematous intimal swelling found in the ascending aorta of one dog seems to be of special interest in this respect, as similar intimal reactions of the aorta were observed among other arterial changes in several dogs which had been given intravenous injections over periods of several months of solutions of S-methylisothiourea sulfate (2-methyl-2-thio-pseudourea) and which showed considerable retention of glycogen in the liver cells even after fixation in solution of formaldehyde, while rabbits similarly treated exhibited a structure of their liver cells resembling that of vegetable cells because of a high glycogen content (Hueper and Ichniowski [1b]).

The observations reported as well as those made recently by Ball concerning the arrest of neoplastic growths in rats receiving repeated intravenous injections of glycogen solution (Pfanstiehl, chemically pure) provide sufficient evidence in support of the concept that the repeated introduction of appreciable amounts of glycogen by the intravenous route is not a harmless procedure as asserted by Bucher.[2d]

SUMMARY

A solution of glycogen injected intravenously into dogs in single or in repeated doses elicits hematic reactions characteristic of the macromolecular hematic syndrome (primary transitory leukopenia, secondary myeloid leukocytosis, anemia, accelerated erythrocytic sedimentation, increased clotting time).

The livers of such dogs show hydropic swelling and fatty infiltration of the liver cells and a negligible amount of glycogen. The aorta and the myocardial and renal arteries exhibit intimal and medial lesions of a proliferative and degenerative nature.

Repeated intravenous introduction of glycogen is not a harmless procedure.

SUDDEN DEATH OF A YOUNG ATHLETE

WITH AN EXCESSIVE CONCENTRATION OF EPINEPHRINE-LIKE SUBSTANCES IN THE HEART MUSCLE

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The case to be reported appears significant as an example of a chemical mechanism causing sudden cardiac death.

B. M. was a 21 year old university student, a senior, prominent in athletics (chiefly track). Both parents and one brother were alive and well. He had undergone the usual childhood diseases. He had not had scarlet fever. Repeated medical examinations revealed excellent health. For three years prior to his death the blood pressure readings were: 135 systolic and 65 diastolic; 124 systolic and 76 diastolic; 112 systolic and 80 diastolic; 145 systolic and 80 diastolic. The pulse rates for the same years were 48, 76 and 65. The heart appeared normal on examination. The pulse rate after exercise was 96 per minute and stayed unchanged at 96 for two minutes or more. In August 1942 during a baseball game he suddenly grew pale, trembled, perspired profusely, complained of feeling sick and had to sit down for a while. He did not smoke or drink and led a sober, regular life. He was very popular among his colleagues.

On Oct. 8, 1942, in the evening, he complained of being tired. He went to bed at 11:30. At about 5:30 the following morning unusually heavy breathing was heard by several of his roommates from the direction of his bed. At 7 a. m. he was found dead, lying on his back in a natural position, with the bed clothing undisturbed.

The autopsy was carried out by Dr. Charles F. Whitney, director of the Vermont State Department of Public Health. His report reads as follows:

"The body of B. M. was 6 feet 1 inch (185.5 cm.) in height and was said to weigh 180 pounds (81.5 Kg.). There were no injuries of any description on the exterior or the interior of the body, which was well developed, with musculature above the average. Nutrition was good. Postmortem rigidity was beginning, and there was little postmortem lividity. The pupils were equal and measured from 7 to 8 mm.

"The interior of the mouth and the upper portion of the throat were not examined except to exclude the presence of any foreign material in the larynx. The thymus was atrophied to an apparent normal degree. The pleural cavity was empty and the lining normal in appearance. The left lung weighed 13 ounces (368.5 Gm.); the air content was slightly lessened; the tissue was a little darker posteriorly, with some increase in blood. There was a little colorless fluid in the bronchi. The vessels of the lung when slit open showed no emboli even in the smallest branches.

"The right lung weighed 14 ounces (397 Gm.). Otherwise it was practically the same as the left. The pericardium was normal in appearance and contained a few cubic centimeters of straw-colored fluid. There were no adhesions. The heart weighed 390 Gm. The epicardium was glistening, and the wall was normal in appearance and showed no fibrosis. The cavities contained fluid blood with some tendency to form soft clots. The valves and the coronary walls were normal; the lumens were empty except some portions that contained unclotted blood, as confirmed by microscopic examination. The aorta was slit open from the heart to about 2 inches (5 cm.) below the beginning of the common iliac arteries and showed no abnormality.

"The omentum was of average appearance, showing no congestion or excess fat. The peritoneum was glistening, with no adhesions or excess fluid. The liver weighed 4½ pounds (2,041 Gm.) and appeared normal in every respect. The gallbladder was of average size and fairly well filled with fluid normal-appearing bile. The spleen weighed 7 ounces (198.5 Gm.). It was slightly darker than normal, appearing to be slightly engorged. The right kidney weighed 6 ounces (170 Gm.), and the capsule stripped easily, showing a glistening surface beneath. It was of normal consistency but appeared to be a little darkened in color. The left kidney was similar to the right except that it appeared to be a little darker, indicating some congestion. Both adrenal glands were normal in size, section surface and general appearance. The bladder contained about one-half ounce (15 cc.) of urine. The prostate gland was not enlarged.

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"The stomach was normal in shape; the walls and lining were likewise normal. It contained about 3 ounces (85 cc.) of mushy material, including mucus and bits of orange pulp and apple skin. The mesentery, the pancreas and the duodenum were normal. In the ileum there was an occasional reddish area 1 to 3 inches (2.5 to 7.5 cm.) in length, which on being sectioned showed some reddening of the wall and of the content, which was of an apple sauce consistency. The large intestine and the appendix were normal.

"The brain coverings were normal and showed no edema, and the brain itself was normal throughout, including the pons and the medulla. No examination of the spinal cord was made. No bacterial examinations were carried out.

"Histologic examination of the tissues showed nothing of a pathologic nature; chemical examination of organs for alcohol and poisons likewise gave negative results."

The only striking abnormal finding in this case of sudden death was an excessively high concentration of epinephrine-like substances in the heart muscle (1,622 color units per gram with a "specific ratio" of 1:0.9).

This result was obtained with a modification of Shaw's colorimetric method for the determination of "epinephrine" in blood and tissues (Raab [1h]). In recent investigations it was found by me [1d] that, besides epinephrine proper and sympathin, epinephrine-like substances with a catechol nucleus, such as adrenalone, dihydroxyphenylalanine, epinine and leukoadrenochrome, participate in the reaction, and also ascorbic acid, which forms only a minor fraction of the colorimetric readings, however. For brevity's sake the findings will be designated by the term "epinephrine-like catechols." [3] According to Tainter [2] and others the catechol

Epinephrine-Like Catechols in the Heart Muscle

Species	Normal Average, Color Units per Gram	Maximum Concentration Compatible with Life-Maintaining Function of Heart, Color Units per Gram
Rat	946 (30 hearts)	1,900
Man	698 (21 hearts)	1,000

ring is responsible for the sympathomimetic actions of a number of substances of this group. Sympathomimetic amines with a catechol nucleus are characterized, furthermore, by a high electrical potential (Ball and Chen [4]).

The excessively high concentration of epinephrine-like catechols in the heart muscle in the case described appears to be of decisive importance as the probable cause of death, for the following reasons:

1. In experiments on rats I found that the heart muscle shows an outstanding tendency to accumulate injected epinephrine and similar catechols, as well as the spontaneously discharged hormones of the adrenal medulla and sympathin—e. g., during physical exercise or exposure to cold.

Cardiac death occurred inevitably after injection of epinephrine when the amount of epinephrine-like catechols in the myocardium exceeded a certain concentration (table).[1c, h]

1. Raab, W.: (*a*) Endocrinology **28**:325, 1941; (*b*) **29**:126, 1941; (*c*) **29**:564, 1941; (*d*) **32**:226, 1943; (*e*) Am. Heart J. **24**:365, 1942; (*f*) Arch. Int. Med. **68**:713, 1941; (*g*) J. Clin. Endocrinol. **1**:977, 1941; (*h*) Exper. Med. & Surg. **1**:188, 1943; (*i*) unpublished data.

2. Tainter, M. L.: J. Pharmacol. & Exper. Therap. **40**:43, 1930.

3. In previous publications (see footnote 1 *a, b, c, f, g*) I interpreted the results obtained with this method as "adrenocortical compounds." This conception was later abandoned when it was found that no actual compound formation of steroids with epinephrine takes place, although the chromogenic and biologic properties of epinephrine are altered by cortical steroids.[1d, e] In articles published elsewhere by me epinephrine-like catechols have been referred to as AC substances (adrenalin-like adsorbable catechols).

4. Ball, E. G., and Chen, T. T.: J. Biol. Chem. **102**:691, 1933.

2. Abnormally high concentrations of epinephrine-like catechols or of epinephrine proper and sympathin were found by me in the majority of the hearts of 33 persons who had died from cardiac failure, particularly in cases of arterial hypertension, angina pectoris, uremia and tumors of the adrenal glands.[1h] This fact seems to correspond with the observation that in arterial hypertension [1f] and angina pectoris [1g] the discharges of adrenal hormones are at times abnormally intense and that there is a permanent accumulation of epinephrine-like catechols in the blood of uremic patients.[1f, i]

3. The fundamental role of sympathomimetic amines with a catechol nucleus in the genesis of myocardial disease is further stressed by an abundance of observations regarding the experimental reproduction of the characteristics of myocardial disease (arrhythmia, anoxic changes of the electrocardiogram, anginal pain, pulmonary edema, hypertrophy and dilatation of the heart, myocardial degeneration and necrosis, cardiac failure and death) through the administration of epinephrine and related amines.[1h] Furthermore, the metabolic peculiarities of the failing human heart (anoxia, loss of creatinine, phosphagen and adenylpyrophosphoric acid) are essentially identical witih the metabolic effects produced in the heart muscle through experimental administration of epinephrine.[1h]

4. Certain hormonal and nutritional factors which were found by me to enhance the accumulation of epinephrine-like catechols in the heart muscle of the rat, such as an overdose of thyroxin or of desoxycorticosterone acetate [1c] and vitamin B_1 deficiency,[11] are known to precipitate cardiac failure in corresponding clinical conditions.

With these facts in mind, the observation of an excess of epinephrine-like catechols in the heart muscle, as in the case described, will be briefly considered in regard to the problem of sudden "unexplained" death.

One of the manifestations of acute toxic epinephrine action on the heart is ventricular fibrillation (Levy and Lewis [5]; Rothberger and Winterberg [6]). Ventricular fibrillation is believed to be a common cause of sudden cardiac death (McWilliam [7]; Nathanson [8]; Weiss [9]). Sudden death has often been brought about in animals by injection of epinephrine hydrochloride and in human beings severe cardiac episodes and death have been observed after injection of this substance. In an experiment which I carried out on myself, then at the age of 32, the subcutaneous injection of 1.3 mg. of epinephrine hydrochloride was followed by severe anginal pain, collapse and acute dilatation of the heart. In another case which came to my knowledge death occurred almost immediately after a subcutaneous injection of an unintentionally high dose (several milligrams) of epinephrine hydrochloride.

Sudden death is comparatively common in persons with pathologic changes of the adrenal glands, such as pheochromocytoma (Tilmann [10]; Paul [11]; Wickenhäuser [12]; Minovici and Bonciu [13]; Holtz [13a]), tumor metastases (Simpson [14]),

5. Levy, A. J., and Lewis, T.: Heart **3**:99, 1912.
6. Rothberger, C. J., and Winterberg, H.: Arch. f. d. ges. Physiol. **142**:461, 1911.
7. McWilliam, J. A.: Brit. M. J. **2**:215, 1923.
8. Nathanson, M. H.: Arch. Int. Med. **58**:685, 1936.
9. Weiss, S.: New England J. Med. **223**:793, 1940.
10. Tilmann, O.: Beitr. z. path. Anat. u. z. allg. Path. **95**:60, 1935.
11. Paul, F.: Virchows Arch. f. path. Anat. **282**:256, 1931.
12. Wickenhäuser, K.: Deutsche Ztschr. f. d. ges. gerichtl. Med. **31**:97, 1939.
13. Minovici, N., and Bonciu, C.: Bull. Acad. de méd. de Roumanie **1**:638, 1936.
13a. Holtz, E.: Deutsche Ztschr. f. d. ges. gerichtl. Med. **32**:94, 1939.
14. Simpson, C. K.: Lancet **1**:851, 1937.

infection (Simpson [14]; Marcos [15]), cystic degeneration and hemorrhages (Kruse [16]; Simpson [14]; Severn [17]; Henderson and Pettigrew [18]; Damon [19]) or even only round cell infiltration and edema (Brack, Plessmann and Krüger [20]).

Sudden deaths without any obvious organic background have been observed under conditions which are known to be accompanied by hormonal discharges of the adrenal medulla or the peripheral sympathetic nerve terminals, such as emotional shock (Markowitz [21]; Bohrad [22]; Brisard [23]; Martin and Royo Villanova y Morales [24]; Stefan [25]; Levy and Bruenn [26]) or strenuous physical exercise (Brack, Plessmann and Krüger [20]; Weyrich [27]; Kitchin [28]; Marradi-Fabroni [29]; Böhmig [30]; Mangili [31]).

Enforced physical exercise was found in animals to be accompanied by an increase of the myocardial concentration of epinephrine-like catechols (Raab [1h]), by cardiac hypertrophy and even by appearance of disseminated foci of myocardial degeneration [1h] similar to those produced experimentally with epinephrine and to those which were observed as the only organic lesion in the hearts of persons whose deaths were sudden and unexplained (Nathanson [8]; Markowitz [21]; Helwig and Wilhelmy [32]; Lisa [33]; Grey [34]). The clinical electrocardiographic and roentgenographic evidence of acute "heart strain" in athletes, soldiers and laborers is more impressive than that of chronic heart strain (Parsonnet and Bernstein [35]). This may be due to the diminishing effect of systematic training on those myocardial metabolic changes which occur during strenuous work (Schumann [36]). Nevertheless, postmortem studies have revealed myocardial hypertrophy in half of a series of 35 hearts of athletes (Kirch [37]). An increased blood level of epinephrine has been observed during athletic activities by Meythaler and Wossidlo.[38]

The most famous case of sudden death of an apparently healthy athlete from "exhaustion" occurred two thousand four hundred and thirty-three years ago when the Marathon runner collapsed, dead, on his arrival in Athens, after having shouted the message of victory over the Persians. It appears most probable from present knowledge that his heart succumbed to ventricular fibrillation due to an acute excessive accumulation of sympathomimetic amines in the myocardium.

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38. Meythaler, F., and Wossidlo, K.: Klin. Wchnschr. **19**:658, 1937.

In the case of the young athlete discussed at the beginning of this article no gross lesions of the adrenal glands or of the sympathetic nervous system were found. A small paraganglioma outside of the glands may have escaped detection, however. In the history only the episode of sudden pallor, trembling and feeling sick during a game two months before death appears significant. The symptoms mentioned correspond exactly to those elicited by an injection or a spontaneous discharge of a toxic amount of epinephrine.

The concentration of epinephrine-like catechols in the heart in this case was not only high above the maximum which had been found to be compatible with the life-maintaining function of the human heart; it was the highest concentration ever observed in a human myocardium so far.

The "specific ratio" [1a, d] of 1:09 indicates that besides epinephrine proper and sympathin a considerable amount of epinephrine-like substances must have been present. Since similar conditions prevail in the adrenal medulla itself (Raab [1d, i]), this finding is well compatible with an adrenal medullary origin of the material found in the heart muscle. An at least partial local origin from adrenergic neurons within the myocardium cannot be ruled out, however.

Death seems to have been preceded by a short period of distress, as suggested by the heavy breathing which was heard by others. No full-fledged pulmonary edema developed, but there were some signs of congestion.

Whether or not the athletic training had contributed to the fatal outcome of this case by gearing the adrenal glands and the sympathin-producing neurons to an exaggerated degree of activity cannot be decided. The occasional moderate elevations of the systolic blood pressure which were recorded during the last years before death and the failure of the pulse rate after exercise to decrease within two minutes suggest a tendency toward a jerky mode of adrenal secretion like that observed in patients with essential hypertension.[1f] Hypertrophy of the muscular apparatus of the adrenal veins, which was found to be a characteristic of such cases (Goldzieher and Sherman [39]; Allen [40]), may be responsible for temporary retentions alternating with sudden releases into the blood stream of unduly large and even fatal amounts of adrenal hormonal material.

SUMMARY

In a case of sudden death of a young, seemingly healthy athlete the only strikingly pathologic change observed at autopsy was an excessively high concentration of epinephrine-like substances in the heart muscle (the highest of all values observed in a series of 54 normal and pathologic human hearts).

From the results of studies of the hearts of animals and man it is concluded that this excessive concentration of sympathomimetic amines was incompatible with survival and was the immediate cause of death.

Chemical examination of the heart muscle may prove to be of forensic usefulness in cases of unexplained sudden death, particularly in those of death occurring under emotional or physical strain.

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STUDIES ON THE HEMOLYTIC STREPTOCOCCUS

VI. COMPARISON OF EXPERIMENTAL LESIONS BY TOXINS OF THE STREPTOCOCCUS OF SCARLATINA WITH THOSE IN FULMINATING SCARLATINA

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Two types of nephritis occurring during the course of, or following, scarlet fever have been described, namely, acute nonsuppurative interstitial nephritis, which is associated with a fulminating infection, and acute glomerulonephritis, which appears about three weeks after the rash and which subsequently may give rise to progressive, so-called chronic interstitial nephritis. The factors responsible for the initial renal damage in scarlet fever are not known nor has the relation, if any, between the two types of nephritis been determined. Interest has centered mainly about the genesis of the glomerular nephritis, which is commonly believed to be dependent on the development of allergic phenomena.[1] The earlier view expressed by Pearce[2] was that the renal damage is due essentially to prolonged exposure of the kidney to toxins.

Experimental work on the effects of the scarlatinal streptococcus, its bacterial lysates and culture filtrates on the kidneys has given inconclusive results. Duval[3] and Duval and Hibbard[4] claimed to have produced in animals nephritis identical in all essentials to scarlatinal nephritis in man by injecting peritoneally produced bacterial lysates, although their results with bacterial injections were negative. Other investigators have failed to verify the results reported by them.[5]

The introduction into animals of streptococcic products with their associated heterogeneous protein mixtures has tended to mask the specific effect of the erythrogenic toxin of the scarlatinal streptococcus on the kidneys. Hückel[6] produced marked degenerative lesions in kidneys of rabbits by injection of Dick toxin into the renal vein and less marked changes by intravenous and by subcutaneous injection. Ahlström[7] likewise injected Dick toxin into rabbits but failed to confirm the work of Hückel.

The separation by Stock[8] of an erythrogenic toxin with a potency of 200,000,000 skin test doses per milligram of protein, which has been shown by electrophoresis[9] to be relatively pure, made possible the investigation of the effect of the toxin

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on the kidneys. In the following pages are outlined the pathologic changes produced in the kidneys of albino mice and golden hamsters following the injection of single and multiple doses of the Stock toxin, of nucleoprotein derived from bacterial filtrates,[10] of combinations of toxin and nucleoprotein derived from the NY5 strain of Streptococcus haemolyticus and of killed Str. haemolyticus. The analogous pathologic changes found in the kidneys of 5 children dying from fulminating scarlet fever are also described.

EXPERIMENTAL PROCEDURE

Mice and hamsters were used for this study because of their small size, which made the injection of relatively large amounts of toxin per unit of body weight more convenient. The kidneys of these two species differ somewhat in their minute structure. In the mouse the capsular space is lined with columnar epithelium in contradistinction to the flattened epithelium obtaining in the hamster. The juxtaglomerular bodies are less prominent in the latter animal. Sixty mice with 10 controls and 59 hamsters with 13 controls were used. The animals were of inbred stocks raised in this laboratory and were 2 to 3 months of age. They were free from infection and showed no evidence of spontaneous nephritis.

The control animals used throughout showed no renal lesions comparable to those produced in the experimental animals.

The mice were divided into three groups. The animals of group 1 received single doses of 1,000 to 1,000,000 skin test doses of toxin subcutaneously and were killed twenty-four, forty-eight, seventy-two hours or a week after the injection. The second group received multiple doses of toxin and were killed twenty-four hours after the last injection. Group 3, consisting of 16 animals, was given nucleoprotein or mixtures of nucleoprotein and toxin.

Three groups of hamsters were studied. The first group, consisting of 5 animals that received 5,000 or 10,000 skin test doses, was killed at twenty-four hours. The results were clearcut and single injections on more animals were not repeated. Group 2, consisting of 30 hamsters, received 5,000 to 1,200,000 skin test doses of toxin in varying total doses of 10,000 to 100,000 skin test doses over periods of two days to three months. In 4 of these animals the toxin was injected into a subcutaneous agar focus in an attempt to insure continuous slow absorption. Group 3, 12 hamsters, received suspensions of killed scarlet fever streptococci over one to three month periods, and 10 received nucleoprotein derived from bacterial filtrate[10] over the same interval. The suspension of streptococci was prepared by washing a forty-eight hour blood agar growth of the NY5 strain with 0.5 per cent phenolized saline solution. The preparation contained approximately 3,000,000 organisms per cubic centimeter, and sterility was determined by culturing the suspension before injection.

The animals were stunned by a blow on the head; the kidneys were removed at once and fixed for twenty-four hours in Zenker's or Zenker-formaldehyde solution.[11] Paraffin sections were cut at 4 microns and stained with hematoxylin and eosin and with the Masson trichrome stain as modified by Lillie.[12] The latter stain proved valuable in bringing out early tubular damage.

RESULTS

Kidneys of Mice.—The kidneys of animals in group 1 that were killed twenty-four hours after the injection of toxin showed no gross changes. Microscopically, the renal damage was mainly degenerative, the degree of damage being proportional to the amount of toxin given. The outstanding change was a rapid disappearance of many glomeruli and disintegration of their subtending tubules, which was apparent with even the small dose (fig. 1 *A*). Approximately two thirds of the renal nephrons could be seen in the process of disappearance. The evanescent character of the lesion was seen in the absence of subsequent fibrosis. The remaining glomeruli were congested and swollen, often with considerable reduction in size of the capsular space. The epithelium showed swelling and vacuolation with either pyknotic or karyolytic nuclei. Leukocytes were little, if at all, increased. The endothelium of the tuft stained deeply and frequently showed piling up of the nuclei in the central part of the glomeruli, which suggested possible beginning hyperplasia. Occasionally, hypertrophied and dividing cells were observed in the capsular epithelium, but it is not believed that this change represented

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11. This is Zenker's solution made with solution of formaldehyde U. S. P. instead of acetic acid.

12. Lillie, R. D.: Stain Technol. **15**:17, 1940.

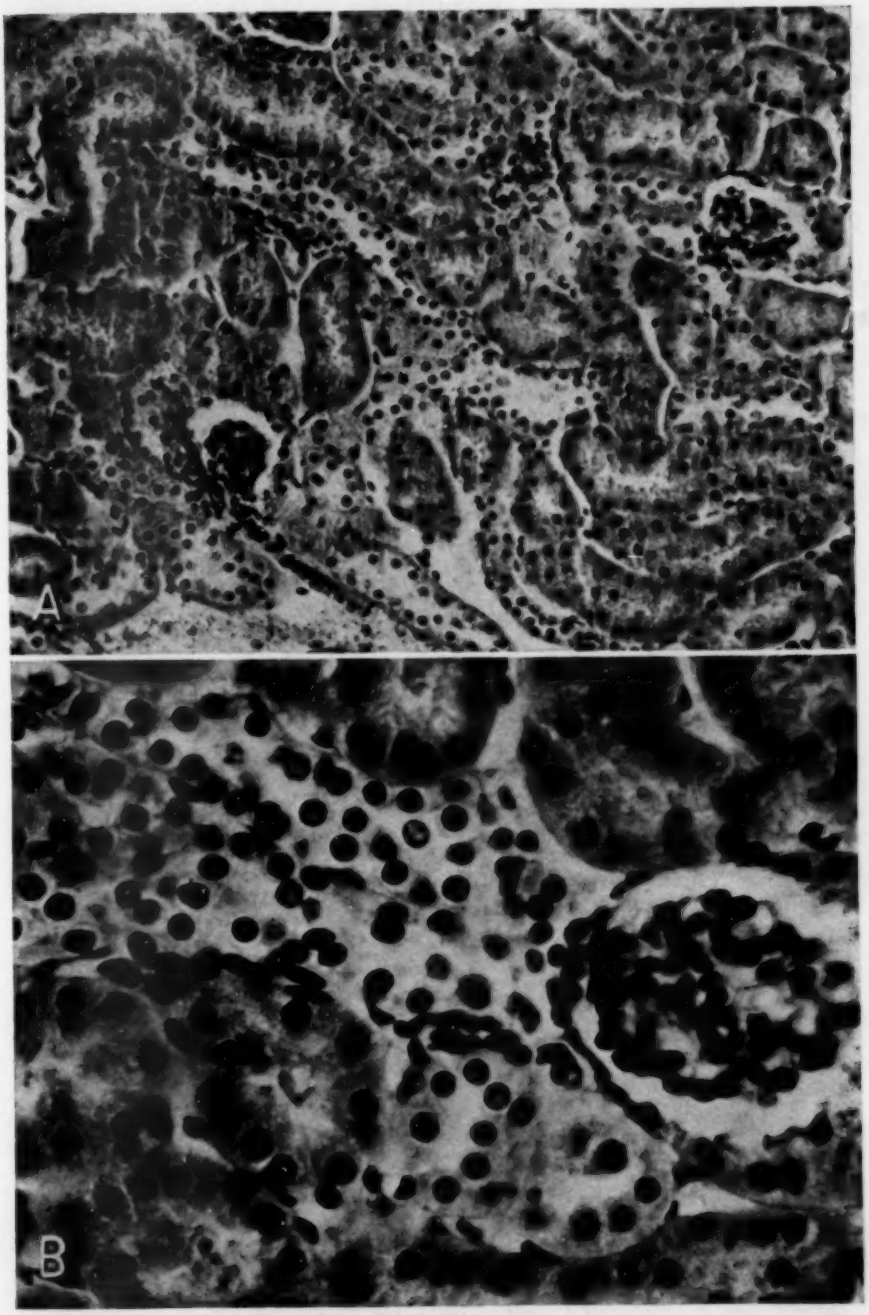

Fig. 1.—*A* (mouse 22), shrunken, disappearing glomeruli and degenerating subtending tubule; hematoxylin and eosin stain; × 200. The mouse had been given 5,000 skin test doses of toxin subcutaneously and was killed in twenty-four hours.

B (mouse 3), glomerulus with columnar epithelium lining the capsular space and varying degrees of degeneration of the subtending tubule; hematoxylin and eosin stain; × 600. This mouse had been given 10,000 skin test doses of toxin subcutaneously and was killed in forty-eight hours.

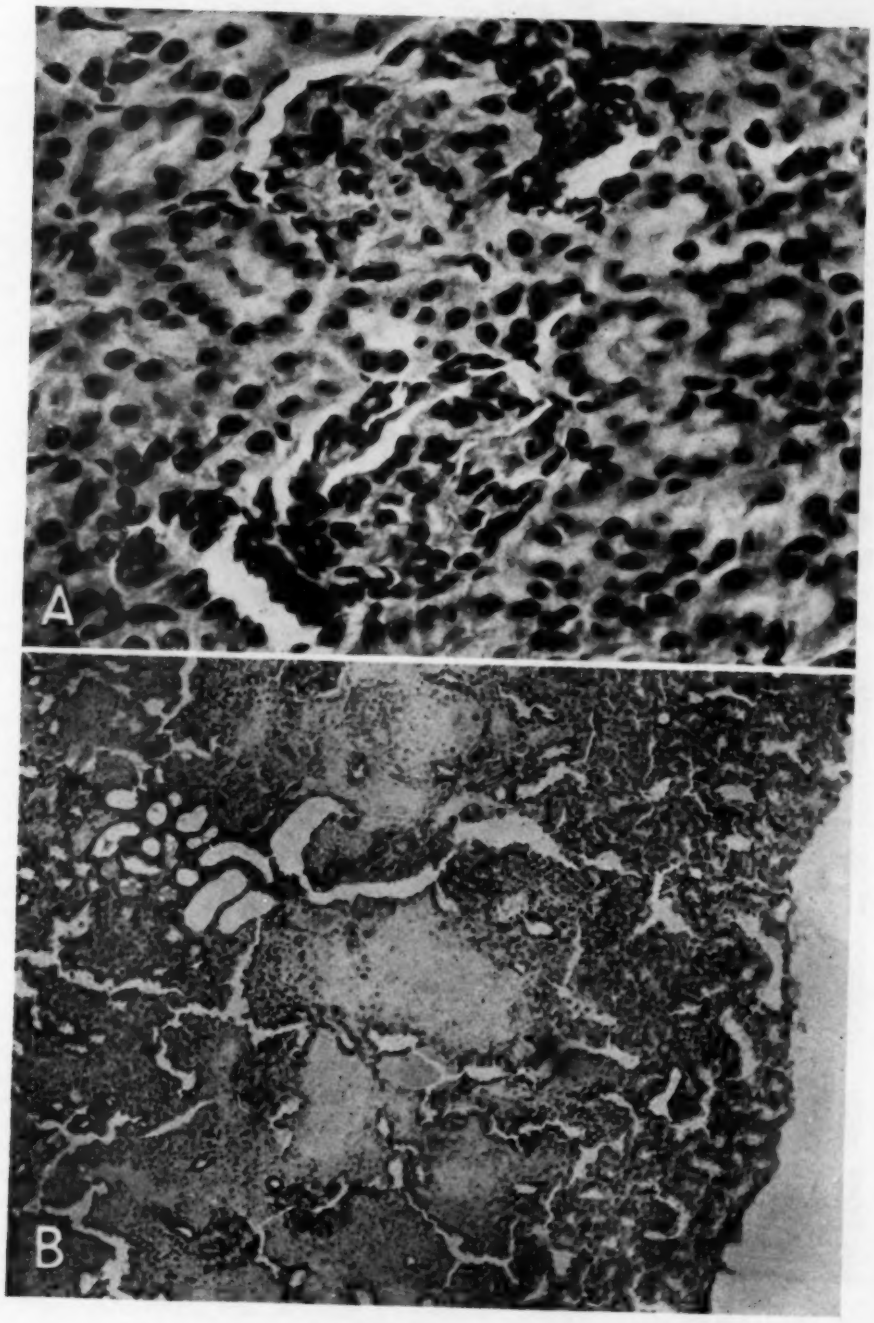

Figure 2

(See legend on opposite page)

early crescent formation. This feature was difficult to evaluate because the columnar cells lining the capsule were frequently cut tangentially. In some places where the glomerulus had completely disappeared the juxtaglomerular body remained apparently intact. The macula densa was seldom recognized. The epithelium of the tubules showed cloudy swelling and varying stages of disintegration and solution. The cell wall often became indistinct early, and the cytoplasm lost its capacity to stain, while the nuclei appeared slightly swollen and resembled early lymphocytes. Animals killed forty-eight, seventy-two hours and a week after single doses of toxin showed the same type of changes, but the degree of degeneration was often more advanced (fig. 1 *B*). The only vascular change noted was possible swelling of the endothelium of the capillaries.

Thirty mice of group 2 were given multiple injections of from 1,000 to 20,000 skin test doses. They received from 2,000 to 220,000 skin test doses over periods of two days to ten weeks and were killed twenty-four hours after the last dose. The kidneys of animals receiving 2,000 to 10,000 skin test doses showed no gross abnormalities. The kidneys of those receiving larger amounts were frequently somewhat swollen and occasionally were pitted. Microscopically, the total number of units affected remained about the same in all the animals, but the degree of degeneration varied according to the amount of toxin used and the time over which it was given (fig. 2 *A*). In addition to the changes described in group 1, the kidneys frequently showed thickening of the basement membrane of the remaining glomeruli and of their capsular lining, thickening of the wall of the afferent vessel, and thickening and splitting of the elastic tissue in the larger arteries. Marked congestion and hemorrhage, particularly among the collecting tubules, also occurred. Compensatory dilatation was present in some of the remaining tubules.

The kidneys of the mice of group 3, which received nucleoprotein in amounts of 0.2 to 2 mg., likewise showed degenerative changes. Fewer nephrons were destroyed in these animals than in those receiving toxin, but the tubular damage appeared more intense. The proximal convoluted tubules remained often as large masses of swollen degenerated tissue staining palely with hematoxylin and eosin and appearing greenish with the Lillie stain, and containing either pyknotic or karyolytic nuclei. The mitochondria showed all stages of degeneration (fig. 2 *B*).

Kidneys of Hamsters.—In general the lesions produced in the kidneys of the hamsters were of the same degenerative character as those described in the mouse.

The reaction to a single dose of toxin (group 1) was, however, acute, as evidenced by the increase in neutrophils in the glomeruli. Otherwise, the pathologic appearance was similar to that in the mouse receiving comparable amounts of toxin.

In group 2 the kidneys grossly varied considerably in appearance. They tended to be increased in size, were granular and dark red. Microscopically, the pathologic change was progressive parenchymatous degeneration with dilatation of the tubules. The reduction in number of glomeruli (from 30 to 40 per cent) was less marked than in the mouse. The glomeruli were swollen and congested, and frequently filled the capsular space. The capsular epithelium was occasionally swollen. The nuclei of the endothelium of the tuft were often pyknotic and seemed somewhat increased in number, especially in the central portion. The epithelial cells were swollen and vacuolated, with either pyknotic or karyolytic nuclei. The juxtaglomerular body was less prominent than in the mouse, but the macula densa was more evident. The tubules showed cloudy swelling, disintegration and solution of the lining cells. The most marked degeneration was in the proximal convoluted tubules, with the distal convoluted tubules being less affected. Dilatation of the persisting tubules was marked, particularly in the animals that had received toxin over several months (fig. 3 *A*). This

EXPLANATION OF FIGURE 2

A (mouse 42), two glomeruli with pyknotic nuclei and reduction of capsular space; hematoxylin and eosin stain; × 600. Adjacent to one glomerulus is a juxtaglomerular body and close to the other is a macula densa. Note the parenchymatous degeneration of the tubular epithelium. This mouse had been given seven subcutaneous doses of toxin of 5,000 skin test doses each on consecutive days and was killed twenty-four hours after the last injection.

B (mouse 119), irregularity of cortical outline, shrinkage of kidney tissue, loss of many glomeruli, with remaining glomeruli swollen and filling the capsular spaces, and varying stages of degeneration of the tubular epithelium; hematoxylin and eosin stain; × 110. The pale areas are masses of the swollen degenerating proximal convoluted tubules; the other tubules show either considerable parenchymatous degeneration with pyknotic nuclei or compensatory dilatation. This mouse had received seventeen subcutaneous injections of 0.05 mg. each of nucleoprotein over three months and was killed twenty-four hours after the last injection.

was probably due to the tendency of the tubules to become blocked by the calcium carbonate in the urine of the hamster, as well as by retained protein detritus. Occasional monocytic and lymphocytic infiltration was seen in focal areas, most frequently near the pelvis but also in the cortex. The basement membrane of the capsule showed thickening and there was also hyaline thickening of the walls of the capillaries. Amyloidosis occurred in 3 hamsters. No variations in the pathologic process were noted in those animals receiving the toxin in an agar focus.

The changes in the kidneys of the hamsters receiving suspensions of the streptococci over periods ranging from twelve days to five months (group 3) were well defined. The glomeruli were congested; some were shrunken, while others were about twice the normal size. Marked vacuolation of the epithelium of the tuft and extreme swelling of the capsular epithelium, which assumed a columnar form, with vesicular or pyknotic nuclei, occurred early. In a few glomeruli, an occasional dividing epithelial nucleus was seen in the tuft. Where the capsular lining epithelium had become thinned or desquamated this change was followed by regeneration which in no instance reached the proportion of an epithelial crescent. Granular debris was present in some of the capsular spaces. The juxtaglomerular body early showed some hyperplasia. This hyperplasia was not permanent, and later an increase in hyaline ground substance occurred in this structure. The wall of the afferent vessel and those of the larger blood vessels were thickened with a similar hyaline material beneath the endothelium and in the adventitia. There was thickening of the basement membrane of the tuft and of the capsular space, which became progressively more marked with increase in dosage and time. The cells of the macula densa adjacent to the juxtaglomerular body or the capsular space were hyperplastic, and the nuclei were elongated at right angles to the glomerulus. The tubular epithelium, especially that of the proximal convoluted tubules, was swollen in some areas and disintegrating in others. Progressive dilatation of the tubules was present throughout, with a few areas of focal scarring situated beneath the capsule.

Other Organs in Both Mice and Hamsters.—The other parenchymatous organs were likewise involved in the degenerative process. This was especially evident in the liver and the heart. The hepatic cells were swollen, vacuolated and irregularly granular. There was no endothelial hyperplasia or lymphocytic infiltration. The cells of the heart showed progressive degenerative changes, which began as either swelling or shrinking of the heart muscle cells with loss of cross striation and pyknosis or karyolysis of the nuclei. Occasionally the nuclei assumed a bilobed character suggestive of amitotic division. The cardiac cells gradually disappeared with subsequent fibrotic change.

Autopsy sections of kidneys from 5 children dying in from two to eleven days from fulminating scarlet fever gave an opportunity for comparing the human with the experimentally produced renal lesions. The clinical data on these children are briefly as follows:

1. A girl aged 6 years. Her temperature rose and an erythematous rash developed on Nov. 29, 1937. Her condition became progressively worse, and she died on December 10 with streptococcic septicemia.

2. A girl aged 3 years. A cut on her foot on July 22, 1936 was followed by pain in the knee on July 26 and an erythematous rash on July 28. The child died on August 4 with streptococcic septicemia following traumatic scarlatinal infection.

3. A boy aged 11 months died four days after the appearance of a scarlatinal rash.

4. A girl aged 4 years. A rash developed on April 26, 1936, and death occurred on May 10. This child showed bilateral cervical adenitis, bilateral otitis media and bronchopneumonia. In addition to Str. haemolyticus, Haemophilus influenzae was present in the lungs.

5. A boy aged 2 years. On Dec. 16, 1937 he had a rise in temperature with a sore throat. A rash developed on the following day, and death occurred with terminal bronchopneumonia on December 21.

The similarity between the experimental lesions and those seen in the kidneys of these patients was remarkable. All the degenerative changes with the exception of focal or diffuse acute interstitial nephritis, which occurred in only one of the five kidneys (patient 1), were duplicated in the animals.

The outstanding feature in all of the human kidneys was a loss of approximately 40 to 50 per cent of the glomeruli. Those remaining were swollen and markedly vascular. Dilatation of the tubules, which showed varying degrees of

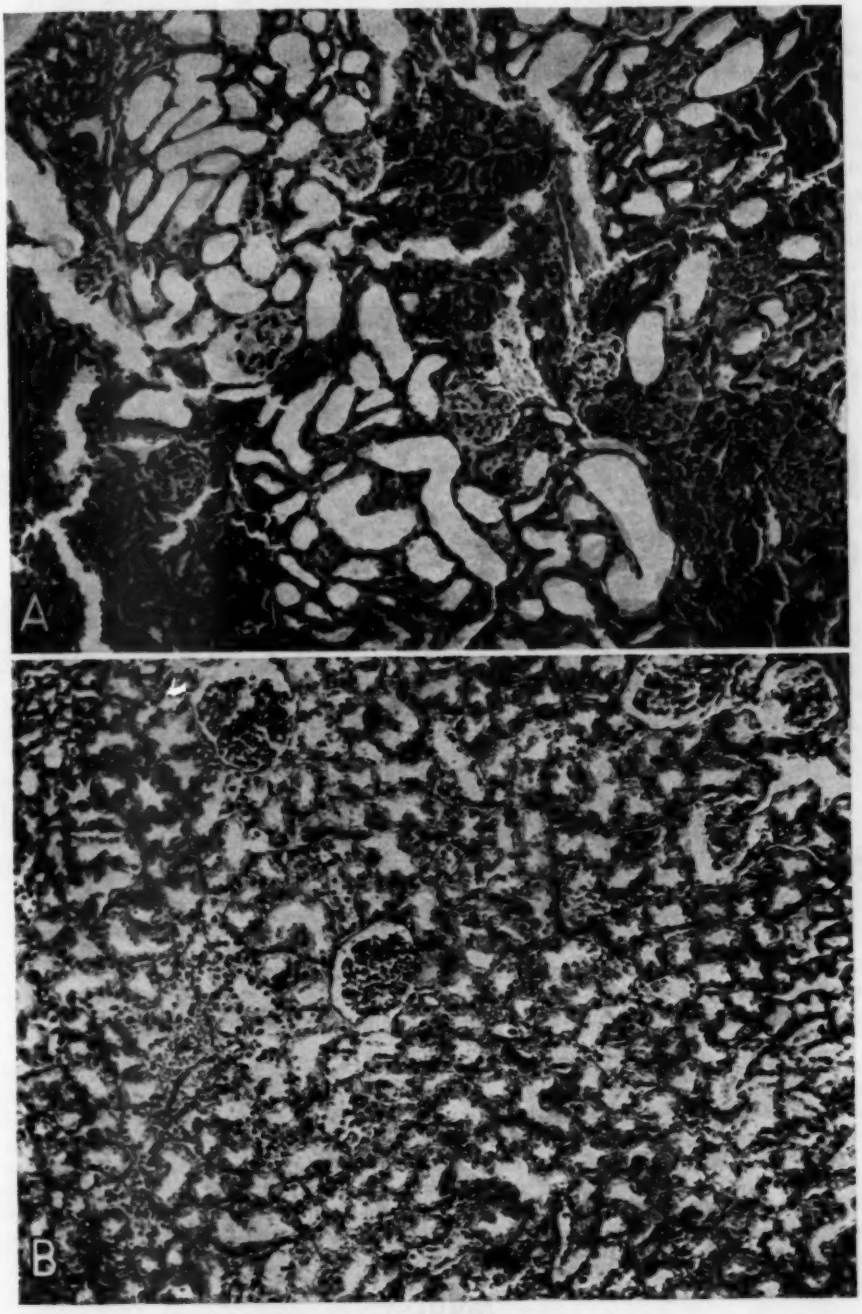

Fig. 3.—*A* (hamster 110), many glomeruli filling the capsular spaces; tubules dilated and epithelial lining degenerated; hematoxylin and eosin stain; × 110. The hamster had received a total of 120,000 skin test doses of toxin in repeated subcutaneous doses of 5,000 skin test doses each over ten weeks.

B (kidney from a child who died of scarlet fever [patient 4]), swollen glomeruli and marked degeneration of the lining epithelium of the tubules; hematoxylin and eosin stain; × 110.

parenchymatous degeneration as well as patchy hemorrhage throughout, was present (fig. 3*B*).

As the five kidneys were practically identical in pathologic appearance, typical findings in the kidneys of patient 1 alone will be described. The epithelium of the tuft showed marked vacuolation, and there was moderate hyperplasia of the endothelium as well as dilatation of the capillaries. Neutrophils were not increased. An occasional glomerulus was swollen, due to congestion and possible endothelial hyperplasia, so that the capsular space was reduced. The latter contained a small amount of granular material. The lining of the capsule was flattened, and there was slight thickening of the basement membrane of the tuft and the capsule. The afferent vessel showed moderate hyaline thickening of the wall with some reduction of the lumen, and there was a questionable increase of intercellular hyaline material in the juxtaglomerular body. Many of the tubules showed marked cloudy swelling, and in places the epithelium of the tubules, especially that of the proximal convoluted ones, was disintegrated

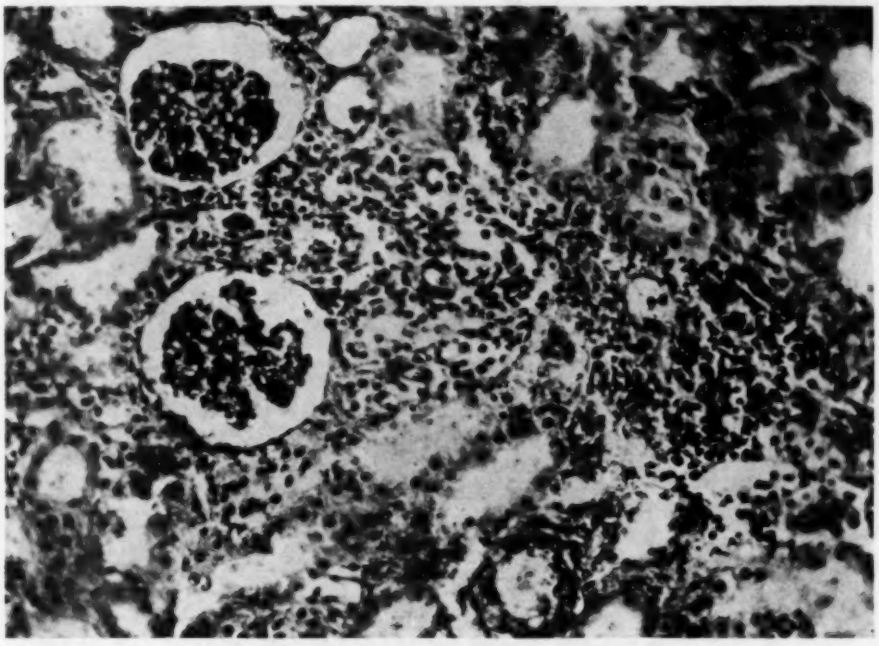

Fig. 4 (kidney from a child [patient 1] who died of scarlet fever).—An area of focal nonsuppurative interstitial nephritis lying adjacent to and below two glomeruli; varying degrees of degeneration of the remaining tubules; hematoxylin and eosin stain; × 200.

and represented either by long rows of pyknotic nuclei embedded in deeply staining cytoplasm or vesicular nuclei with little or no cytoplasm. There was massive degeneration in scattered areas infiltrated with a mixture of these two types of cells, giving a typical picture of focal nonsuppurative interstitial nephritis (fig. 4). Nuclei in these areas frequently gave the impression of collections of lymphocytes, but close examination demonstrated that few if any lymphocytes were present. The nature of these cells could be ascertained by studying individual tubules which showed the same type of degeneration with shrunken or pale, swollen cytoplasm and pyknotic and karyorrhetic nuclei. Irregularly distributed areas of hemorrhage occurred throughout, but most frequently among the collecting tubules.

COMMENT

The injection of erythrogenic toxin of the NY5 strain of Str. haemolyticus into mice and hamsters produced degenerative changes in the kidneys and in other parenchymatous organs, the extent of which depended on the amount given and the time over which the toxin acted. In the kidney 30 to 70 per cent of the nephrons were affected to some degree by a single adequate dose. The degenera-

tion in the glomeruli was most readily demonstrable in the swelling and vacuolation of the epithelium of the tuft, while the endothelial cells were frequently shrunken. The degenerative changes in the tubules were always striking. During the first ten days there were progressive disintegration and sloughing of the tubular epithelium. After this acute phase, during which the kidney apparently offered little resistance to the toxin, the animal seemed to develop resistance or tolerance toward further doses of the toxin, as there was little further destruction of nephrons. The mechanism that underlay this resistance is not clear. It may have been a local resistance of the unit to the toxin or it may have been dependent on formation of antibodies. Morphologically, there was concurrent hyaline-like thickening of the basement membrane of the tuft and the capsular epithelium, as well as of the wall of the afferent vessel which might have influenced resistance toward the toxin. The lesions obtained were identical with those produced by Hückel with comparable amounts of toxin except that no evidence of epithelial crescents was found. Focal lymphocytosis was observed in only a few kidneys and an acute interstitial nonsuppurative lesion, either localized or diffuse, such as was seen in the kidneys of 1 of 5 children who died of scarlet fever was not produced. None of the more chronic lesions described by Duval and Hibbard were observed even in those animals that had received toxin over several months.

The kidneys from all the experimental animals gave a typical picture of acute nephrosis without evidence of nephritis.

The degenerative changes found in the kidneys of the 5 children who died of fulminating scarlet fever followed the same pattern as those in the animals. One showed so-called focal interstitial nonsuppurative nephritis. Variations in the intensity of the lesions were more marked in the clinical cases, and accelerated intensity of the process may be the explanation for an occasional acute interstitial reaction. The acute interstitial reaction was a rapid massive breakdown of tissue containing vesicular and pyknotic nuclei of degenerated tubular epithelium with a few neutrocytes and few or no lymphocytes. The appearance of these lesions observed by us agree in material detail with those described by Councilman,[13] but our studies do not support the interpretation offered for the origin of the component cells. The majority of these cells undoubtedly were derived from tubular epithelium which was undergoing varying degrees of degeneration, but they were so crowded together that they appeared to be intertubular in distribution. The degenerative change in the glomeruli embedded in these focal areas may have been overlooked by Councilman. The degenerative changes undoubtedly were the result of the direct deleterious action of the toxin. It would appear that the late renal lesions developing subsequent to scarlet fever are due to depletion of remaining units rather than to chronic glomerulonephritis.

SUMMARY

The erythrogenic toxin elaborated by the NY5 strain of Str. haemolyticus when injected subcutaneously in a single dose or in multiple doses produces degenerative lesions in the kidneys of mice and hamsters characteristic of acute nephrosis. Focal lymphocytosis was occasionally produced but never an acute interstitial nonsuppurative lesion. In 5 children who died from fulminating scarlet fever, similar lesions were found, with an added focal nonsuppurative reaction in 1 child.

13. Councilman, W. T.: Am. J. M. Sc. **114**:23, 1897; J. A. M. A. **46**:81, 1906.

ALKALINE PHOSPHATASE LEVEL IN URINE IN RELATION TO RENAL INJURY

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AND

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NEW YORK

While investigations were in progress on the phosphatases in the blood and the urine of patients with prostatic carcinoma, the question arose as to the source of alkaline phosphatase in the urine and its relation to disease.

This enzyme is present in large amounts in the proximal convoluted tubules of the kidneys of man, the dog, the rabbit and other mammals.[1] The distal convoluted tubules and the collecting tubules contain no enzyme. Gomori [1b] stated that only in man and, less regularly, the dog does the ascending limb of Henle's loop contain phosphatase. He found that the transitional epithelium of the urinary passages of all species examined contained this enzyme. The level of this enzyme in normal human serum ranges between 5 and 10 King-Armstrong units per hundred cubic centimeters.[2] In human urine its level varies between 0 and 1.5 units per hundred cubic centimeters.[3]

Few studies have been made on the alkaline phosphatase content of damaged kidneys. Gomori [1b] stated that nephritic kidneys contain only traces of alkaline phosphatase or none. Hepler, Simonds and Gurley [4] produced nephrosis in dogs by the administration of uranium nitrate, potassium bichromate and mercury bichloride and studied the phosphatase contents of the kidneys by chemical and histologic methods. None of these poisons inactivated the alkaline phosphatase, although some of the cells containing the enzyme were killed. They observed that necrotic and desquamated tubular epithelium of the kidneys of dogs poisoned by mercury bichloride stained diffusely by Gomori's method for alkaline phosphatase and identified both phosphatase and calcium phosphate in the cellular debris in the terminal portion of the proximal convoluted tubules.

EXPERIMENTAL PROCEDURE

Renal damage was produced in rats by subcutaneous injections of uranium nitrate and in rabbits by intravenous injections of this drug.

Albino rats between 2 and 5 months of age and weighing from 160 to 360 Gm. were used. They were kept in metabolism cages constructed for this purpose; this enabled the collection of urine uncontaminated by feces, food and drinking water. The albino rabbits used weighed from 3,300 to 4,600 Gm. and were between 6 and 9 months of age. They were kept in a cage similar to that employed for the rats.

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1. (*a*) Gomori, G.: Proc. Soc. Exper. Biol. & Med. **42**:23, 1939; (*b*) J. Cell. & Comp. Physiol. **17**:71, 1941. (*c*) Kabat, E. A., and Furth, J.: Am. J. Path. **17**:303, 1941.

2. Huggins, C., and Hodges, C.: Cancer Research **1**:293, 1942.

3. Scott, W. W., and Huggins, C.: Endocrinology **30**:107, 1942.

4. Hepler, O. E.; Simonds, J. P., and Gurley, H.: Proc. Soc. Exper. Biol. & Med. **44**:221, 1940.

The acid and the alkaline phosphatase in the serum were determined according to the technic of King and Armstrong [5] as modified by Huggins and Hodges.[2] The method of Scott and Huggins [3] was used for the determination of phosphatase in urine.

All tissues were stained routinely with hematoxylin and eosin and for alkaline phosphatase by a slight modification [1c] of Gomori's method.[1a] All stains for phosphatase were controlled by stains for calcium.

EFFECT OF THE ADMINISTRATION OF URANIUM NITRATE ON THE PHOSPHATASE CONTENT OF URINE

Rat 1, a male weighing 200 Gm., was given 3 mg. of uranium nitrate subcutaneously. Figure 1 shows the alkaline phosphatase per hundred cubic centimeters of urine and the total daily urinary excretion of this enzyme. The concentration of alkaline phosphatase in the urine excreted during the first twenty-four hours following the injection of uranium nitrate rose to 49.5 units per hundred cubic centimeters, about seven times the highest level obtained before the injection. The total excretion of alkaline phosphatase in twenty-four

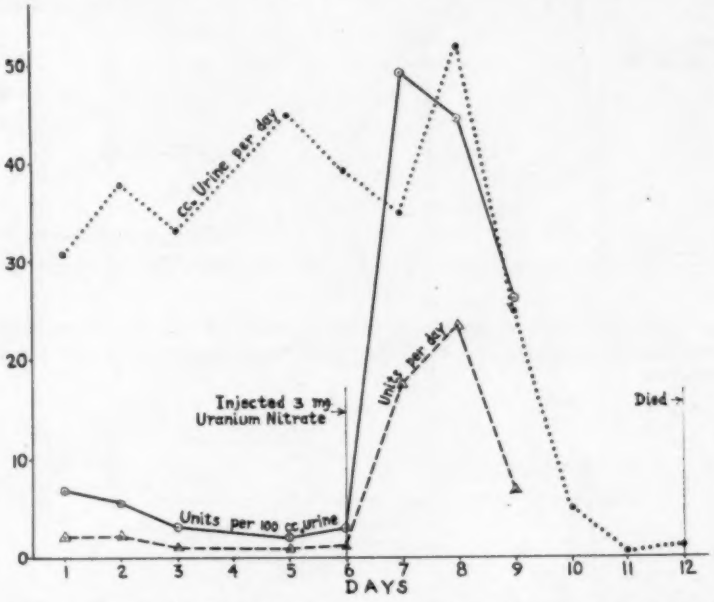

Fig. 1 (rat 1).—The effect of the subcutaneous injection of 3 mg. of uranium nitrate on the urinary output, the concentration of alkaline phosphatase in the urine and the daily excretion of this enzyme. The units are those of King-Armstrong.

hours also increased markedly. This high level of excretion was maintained for forty-eight hours, after which the phosphatase content of the urine dropped precipitously and the urinary volume declined rapidly. The animal died in convulsions six days after the injection.

Rat 2, a male weighing 270 Gm., received 0.5 mg. of uranium nitrate subcutaneously. It survived the immediate renal damage and was killed thirteen days after the injection. The phosphatase levels in the urine are shown in figure 2. Both the concentration of alkaline phosphatase in the urine and the total amount of this enzyme excreted in the urine rose markedly during the twenty-four hours following the administration of the drug. Only after five days did the phosphatase values of the urine return to normal levels.

Rat 3, a female weighing 200 Gm., was given subcutaneously 5 mg. of uranium nitrate. Before the drug was injected the urine contained 2.6 to 4.6 units of alkaline phosphatase per hundred cubic centimeters. In the twenty-four hours following the administration of uranium nitrate the urine contained 40.5 units per hundred cubic centimeters. The total amount of

5. King, E. J., and Armstrong, A. R.: Canad. M. A. J. **31**:376, 1934.

alkaline phosphatase excreted increased from an average of 0.6 unit per day to 4.5 units in the twenty-four hours following the injection and then declined to 3.6 units in the following twenty-four hours. The rat died four days after the injection.

Rat 4, a male weighing 340 Gm., received 4 mg. of uranium nitrate subcutaneously. Before the administration of the drug the daily excretion of alkaline phosphatase varied

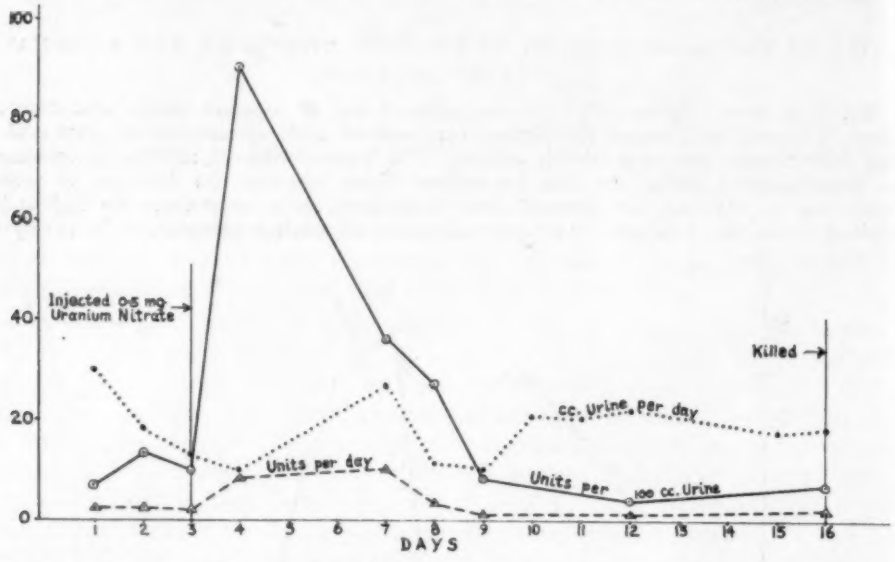

Fig. 2 (rat 2).—The effect of the subcutaneous injection of 0.5 mg. of uranium nitrate on the urinary output, the concentration of alkaline phosphatase in the urine and the daily excretion of this enzyme.

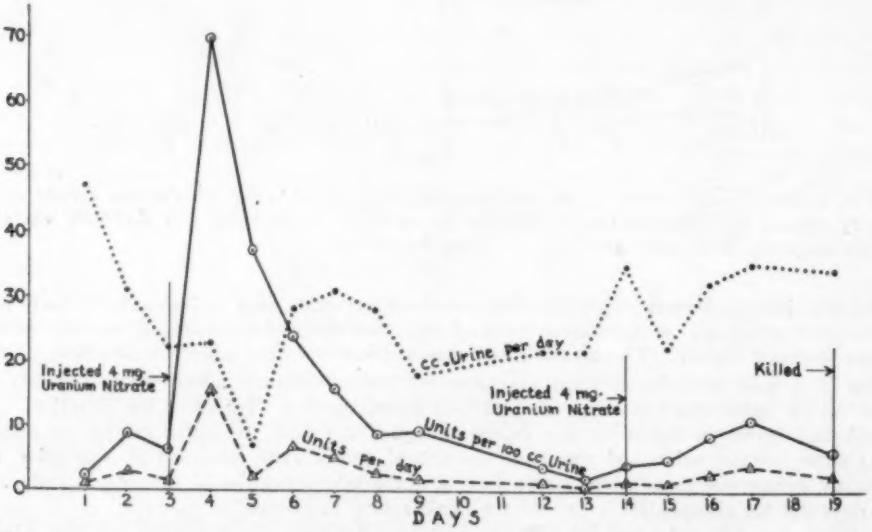

Fig. 3 (rat 4).—The rise in the concentration and total daily amount of alkaline phosphatase in the urine excreted following the first injection of uranium nitrate. A slight delayed rise followed the second injection.

between 2 and 4.8 units per day, while the concentration of the enzyme per hundred cubic centimeters of urine varied between 4 and 15 units. During the twenty-four hours following

the administration of the uranium nitrate the urine contained a total of 26.5 units of alkaline phosphatase, with a concentration of 120 units per hundred cubic centimeters. During the next six days the amount of the enzyme in the urine gradually declined. A second injection of 4 mg. of the drug given eleven days after the first brought about only an inconspicuous and delayed rise in the phosphatase level (fig. 3). The rat was killed sixteen days after the first injection.

Rat 5, a male weighing 260 Gm., was given two doses of uranium nitrate as was rat 4. The first injection likewise caused a sudden increase in the concentration and the amount of alkaline phosphatase in the urine; the second, only a small and delayed rise.

Rabbit 1 was given intravenously 2 mg. of uranium nitrate per kilogram of body weight. The alkaline phosphatase excreted in the urine rose from an average of 4.7 units to 17.0 units in the first twenty-four hours after the injection and to 29 units in the twenty-four to forty-eight hour period. The concentration of alkaline phosphatase per hundred cubic centimeters of urine increased from an average of 6.3 units before injection to 12.3 units per hundred cubic centimeters in the first twenty-four hours and to 22.1 units in the twenty-four to forty-eight hour period. After forty-eight hours this rabbit became anuric and was killed after ninety-six hours.

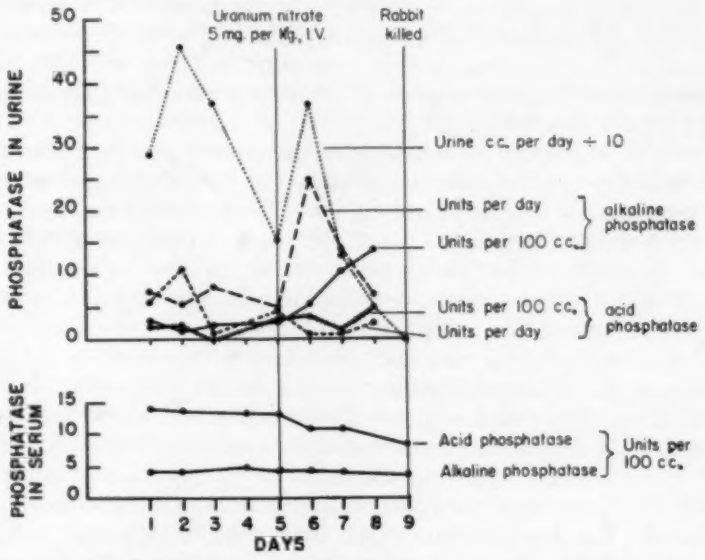

Fig. 4.—The effect of the intravenous injection of uranium nitrate on the acid and the alkaline phosphatase in the serum and the urine of rabbit 2.

Experiments were performed on 2 other rabbits to correlate the blood serum and urinary levels of acid and alkaline phosphatase.

Rabbit 2, a male, was given 5 mg. of uranium nitrate per kilogram of body weight intravenously. The results are shown in figure 4. Before the injection the average twenty-four hour output of alkaline phosphatase in the urine was 6.5 units, with a concentration of 2.3 units per hundred cubic centimeters. In the first twenty-four hours after the injection of uranium nitrate, 24.7 units of alkaline phosphatase was excreted, a concentration of 6.7 units per hundred cubic centimeters. The twenty-four hour output of urinary acid phosphatase and the concentration of acid phosphatase per hundred cubic centimeters of urine were not appreciably altered by the administration of the uranium nitrate. The serum alkaline phosphatase remained the same after the administration of the uranium nitrate, while the serum acid phosphatase decreased slightly. The animal was killed four days after the injection.

Rabbit 3, a female, was given 10 mg. of uranium nitrate per kilogram of body weight. During the first twenty-four hours after the injection no urine was passed. Then the rabbit passed 44.5 cc. but became anuric and was killed on the sixth day. Before the injection the average twenty-four hour output of alkaline phosphatase in the urine was 3.9 units and the

average concentration was 2.8 units per hundred cubic centimeters. The single specimen voided after the injection contained 35.3 units, with a concentration of 82.1 units per hundred cubic centimeters. The twenty-four hour output of urinary acid phosphatase before the injection averaged 0.9 unit and after the injection 0.7 unit. The serum alkaline phosphatase rose gradually from an average of 3.8 units before the injection to 5.8 units forty-eight hours after the injection and 5.7 units before the animal was killed. The serum acid phosphatase averaged 8.2 units before the injection; forty-eight hours after the injection it was 9.1 units, and it was 10.5 units before the animal was killed.

In both rats and rabbits, following the injection of uranium nitrate there was a sharp rise both in the total amount of alkaline phosphatase excreted in the urine and in the concentration of this enzyme. In 2 rabbits tested there was no change in the concentration of the acid or the alkaline phosphatase of the blood or of the acid phosphatase of the urine with the rise of the alkaline phosphatase of the urine.

In 2 rats a second injection of uranium nitrate brought about only an inconspicuous and delayed rise in the excretion of alkaline phosphatase.

Relation of the Phosphatase to Sediment in the Urine.—The urine of 1 rabbit and 2 rats was centrifugated at approximately 2,000 revolutions per minute for thirty minutes. The supernatant fluid was clear and the sediment well packed. Alkaline phosphatase was determined on the supernatant fluid and on the sediment resuspended in saline solution.

In the case of the freshly voided urine of the rabbit (no. 1), passed twenty-four hours after the injection of uranium nitrate, the concentration of alkaline phosphatase in the sediment was five and nine-tenths times that in the supernatant fluid. However, the sediment contained only 21 per cent of the total phosphatase in the urine. The sediment of a sample collected during the next twenty-four hours had a concentration of this enzyme twenty-eight times that in the supernatant fluid and contained 46 per cent of the phosphatase in the urine.

The urine of one of the 2 rats (no. 3) before the injection of uranium nitrate contained a total of 2.9 units of alkaline phosphatase per hundred cubic centimeters; 23 per cent of this was found in the sediment. The concentration of the enzyme in the sediment was sixty-five times that in the supernatant fluid. The urine passed in the twenty-four hours following the intravenous injection of uranium nitrate contained 40.5 units per hundred cubic centimeters, 13 per cent of which was found in the sediment. The concentration of the enzyme in the sediment was twenty and three-tenths times that of the supernatant fluid. The urine of the other rat (no. 5) contained before the injection of uranium nitrate a total of 9.6 units of alkaline phosphatase per hundred cubic centimeters. Eighty-two per cent of this was found in the supernatant fluid, but the concentration of the enzyme in the sediment was forty-eight times that in the supernatant fluid. During the twenty-four hours following the intravenous injection of uranium nitrate the urine contained 29.8 units per hundred cubic centimeters. Twenty per cent of this was found in the sediment, and the concentration of the enzyme in the sediment was twenty-five times that in the supernatant fluid.

It is evident, then, that the sediment of the urine contains a much higher concentration of phosphatase than the supernatant fluid, suggesting that some of the phosphatase remains in sedimentable form, as in casts or in cells. Most enzyme, however, is actually found in the supernatant fluid.

HISTOLOGIC OBSERVATIONS

It is known that small amounts of uranium nitrate cause necrosis of the proximal convoluted tubules; larger doses may damage the loops of Henle and even the

distal convoluted tubules. Oliver [6] has shown that the debris which loosely fills the proximal convoluted tubules after uranium nitrate poisoning largely comes from the necrotic epithelial cells of the tubules themselves. The more condensed casts which fill the lumens of the intact distal convolutions are also formed from the debris of the damaged epithelium of the proximal convolutions.

A microscopic study was undertaken to correlate the chemical changes in the phosphatase content of the urine with the phosphatase content of renal cells as revealed by the histochemical method of Gomori.[1a, b]

The earliest changes were studied in sections from rabbit 1, killed four days after the injection of uranium nitrate. The glomeruli were normal. In about one third of the convoluted tubules the epithelium was necrotic and the tubular space filled with granular eosinophilic cellular debris; in other tubules the epithelial cells were undergoing necrosis. In the remaining third of the tubules the nuclei were normal but the cytoplasm was eosinophilic and granular and the cell borders indistinct. Many of the loops of Henle and some of the collecting tubules were filled with cellular casts composed largely of sloughed epithelium from the proximal convolutions.

In the sections stained for alkaline phosphatase (fig. 5) the damaged and necrotic cells of the proximal convoluted tubules contained this enzyme. The phosphatase was, however, no longer found concentrated in luminal parts of cells but was distributed throughout the cytoplasm. In some places phosphatase-rich cells lay free in the tubules. All of the cellular casts composed of such sloughed cells contained a large amount of the enzyme. The few albuminous casts did not contain phosphatase. The control section contained no calcium.

These observations suggest that the rise of the alkaline phosphatase content of the urine following the injection of uranium nitrate was caused by the excretion of necrotized phosphatase-rich cells of the proximal convoluted tubules.

Rabbit 2 was killed on the fourth day after the injection of uranium nitrate and rabbit 3 on the sixth day. Many of the proximal convoluted tubules and occasionally also loops of Henle and distal convoluted tubules of these 2 rabbits were necrotic.

In both animals parts of the convoluted tubules were lined by regenerating epithelium often containing many mitotic figures. The lumens of many convoluted tubules were filled with sloughed necrotic epithelial cells and with epithelial casts. The kidneys of these rabbits contained much less alkaline phosphatase than normal. Damaged cells of the proximal convoluted tubules contained small amounts of phosphatase. The regenerating epithelium contained none. The debris of necrotic cells in the convoluted tubules and the granular and cellular casts in these and the collecting tubules were rich in phosphatase. A few focal areas of calcification were seen in the cortex of the kidney of rabbit 3.

In rat 1, which died of renal insufficiency six days after the injection of uranium nitrate, there was calcification in many phosphatase-containing casts in the kidney. The glomeruli appeared unaltered. There was profound degeneration and necrosis of most of the convoluted tubules and loops of Henle. Eosinophilic granular and epithelial casts filled many of the convoluted tubules, the loops of Henle and some of the collecting tubules. Some of the casts in the convoluted tubules contained concretions that stained as does calcium.

The alkaline phosphatase stain (fig. 6) showed that the entire system was markedly depleted of this enzyme. The cells of the few undamaged proximal

6. Oliver, J.: Architecture of the Kidney in Chronic Bright's Disease, New York, Paul B. Hoeber, Inc., 1929, p. 100.

convoluted tubules stained pale brown and contained much less phosphatase than normal. The granular casts gave a strong reaction for alkaline phosphatase. The calcified concretions stained deep brown, while the phosphatase granules stained light brown. In the control sections the phosphatase remained unstained.

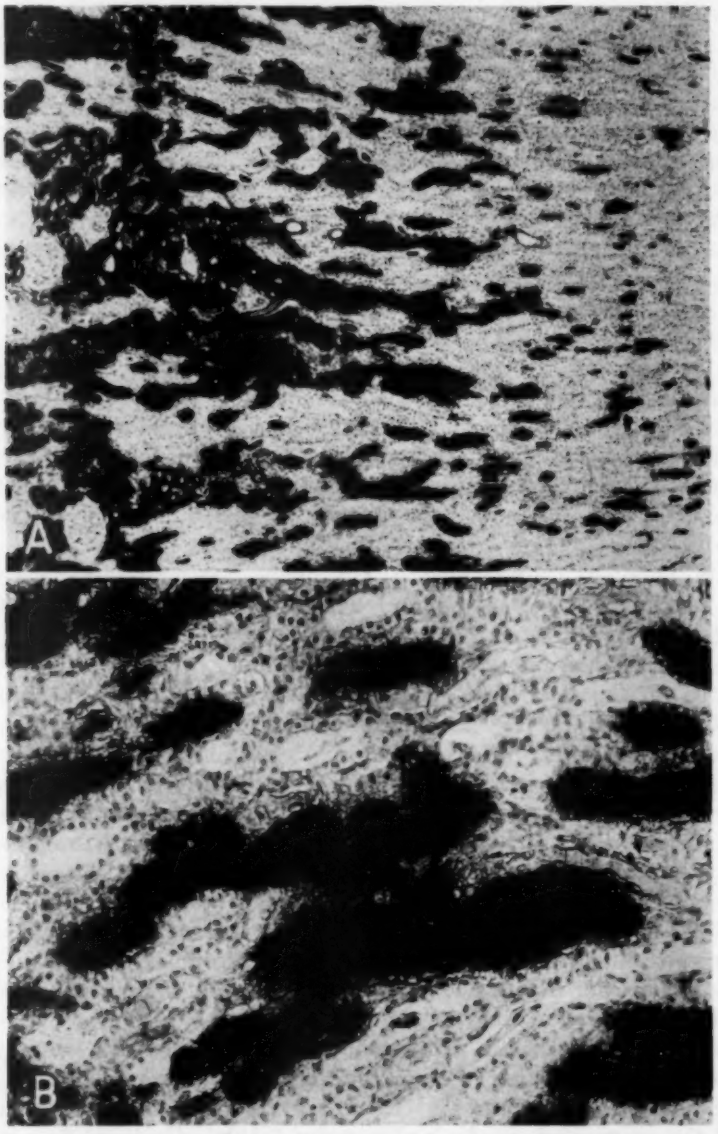

Fig. 5.—*A*, section through the lower part of the cortex and the upper part of the medulla of the kidney of rabbit 1. The black masses are the granular and cellular casts in the convoluted tubules rich in phosphatase; × 60. *B*, high power view of *A*; × 400.

Despite the depletion of the phosphatase in the tubules, the vascular endothelium and the epithelium of the pelvis gave a strong phosphatase reaction. The epithelium of the small intestine also stained strongly for the enzyme. The liver and the spleen showed no histologic evidence of toxic damage.

The microscopic examination of the kidney of rat 2, which survived a small injection of uranium nitrate for thirteen days and seemed well when killed, gave the following results. There were large areas of calcification scattered throughout

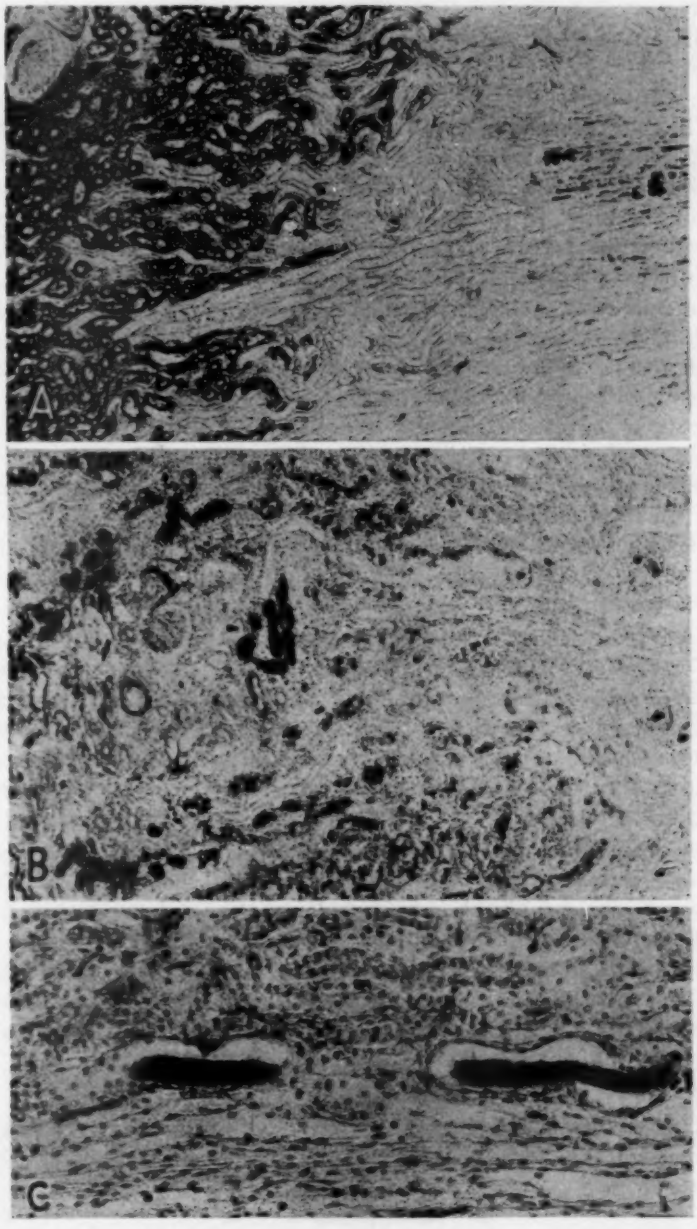

Fig. 6.—*A*, normal rat kidney stained for alkaline phosphatase. The sites of the enzymatic activity stain black; × 60. *B*, kidney of rat 1. The convoluted tubules are strikingly depleted of phosphatase. The large black masses are phosphatase-rich cellular and granular casts; × 60. *C*, high power view of phosphatase-rich casts in the collecting tubules of the kidney of rat 1; × 180.

the cortex with the shape of convoluted tubules. In rat 1 there was slight calcification in the casts; in rat 2 the calcification was marked. Most glomeruli were undamaged; an occasional one was calcified. Some of the convoluted tubules were cystic; others near the areas of calcification were atrophic. There were granular or epithelial casts and many hyaline casts in a few collecting tubules. The normal-appearing proximal convoluted tubules contained a large amount of this enzyme. The cells of the cystically dilated tubules were phosphatase free, while those with either atrophic or regenerating epithelium contained little or no enzyme. The phosphatase reactions of the casts of this kidney were variable. The granular casts, many of which were calcified, contained large amounts of phosphatase, while the homogeneous hyaline casts contained little or none. The epithelium of the renal pelvis and the epithelium of the small intestine contained a normal amount of enzyme. Calcification was observed in the media of numerous arteries in different organs.

Different microscopic changes were seen in the kidneys of rats 4 and 5, which recovered from the effects of the first injection of uranium nitrate and reacted slightly to a second injection, and were killed on the sixteenth and thirty-sixth days after the first injection. The glomeruli were normal except for an occasional adhesion between the glomerular tuft and Bowman's capsule. Only a few small calcific deposits were seen in the cortices. The conspicuous feature was marked cystic dilatation of many of the proximal convoluted tubules with flattening of the epithelium. In their lumens were a few desquamated epithelial cells but no casts. Other tubules were small and atrophic, and only few tubules were normal. In the tubules of the radiating zone and in collecting tubules of the medulla were many hyaline casts.

In the sections stained for phosphatase the cells were markedly depleted of this enzyme. The scattered clusters of apparently normal proximal convoluted tubules contained the usual amounts of phosphatase in their cytoplasm, but their brush borders, which normally are rich in phosphatase, contained none. Epithelial cells lining greatly dilated cortical tubules contained little or no phosphatase. The homogeneous hyaline casts contained none. No reduction of phosphatase was seen in the intestinal epithelium.

Thus the outstanding histologic change in these kidneys was the presence of large numbers of regenerated and damaged tubular cells which contained little alkaline phosphatase. The absence of phosphatase-rich casts in the tubules following the second injection of uranium nitrate correlates well with the failure of the second injection to cause a rise of urinary alkaline phosphatase. MacNider [7] has already shown that after damage by uranium nitrate the regenerated epithelial tissue of the kidney and the liver not only is different morphologically but is more resistant to the same and different toxic substances, such as chloroform, ether and mercury bichloride.

The specific gravity of the urine of each of these 2 rats after the second injection varied between 1.014 and 1.020, indicating that the kidneys did not lose their ability to concentrate urine.

CONTROL OBSERVATIONS

The changes in the output of phosphatase in the urine of the experimental animals appear significant, although the absolute values obtained may have been influenced by variables secondary to or independent of tubular damage. For

7. MacNider, W. B.: J. Pharmacol. & Exper. Therap. **73**:186, 1941.

example, the figures show some rise in the concentration of phosphatase with reduction of urinary output. This, however, is not sufficient to explain the magnitude of the changes observed. The experimental animals had free access to water, and direct observations indicate a fair level of fluid intake. In one experiment, fluid was withheld from a rat for three days, and during this time the alkaline phosphatase content in the urine reached as much as 27 units per hundred cubic centimeters. When the rats were given access to water again, the figures dropped to 5 to 7 units; at the same time the urinary output rose from 3 to 38 cubic centimeters per day. It is evident, however, that the total amount of phosphatase excreted did not rise. The rise of the alkaline phosphatase in the urine following the administration of uranium nitrate usually took place before the decrease in the amount of urine excreted (figs. 1 to 4).

The question arose whether food or feces contaminating the urine may in part have been responsible for the levels of phosphatase obtained. The cages used prevented more than minimal contamination. Analysis of the commercial dog biscuit given as food to the rats showed it to be free from phosphatase. The milk used in feeding the rats was pasteurized, and phosphatase is heat labile. Feces contain large amounts of phosphatase (10 to 80 units per gram of dried feces) but it was obvious from the clarity of the urine obtained as compared with dilute fecal suspensions that the error from this source was small. Uranium nitrate did not produce diarrhea, and the quantity of feces did not change significantly after its administration, while in the case of 2 rats tested the phosphatase values of the feces after the administration of uranium nitrate decreased to approximately one half.

COMMENT

The simplest explanation of excess urinary excretion of alkaline phosphatase after injection of uranium nitrate is that this enzyme is derived from damaged epithelium of the proximal convoluted tubules. Necrotized epithelial cells slough into the tubular lumens, form phosphatase-rich casts and are excreted in the urine.

At least 13 to 46 per cent of the total phosphatase excreted after the administration of uranium nitrate is present in low speed sediments of the urine in the form of casts and other particulate matter. The remaining quantity of excreted phosphatase is not contained in casts or whole cells. The source of this nonsedimentable phosphatase is not known. It may be plasma phosphatase passed by damaged glomeruli or passed through permeable tubular walls. However, there was no coincident change in the alkaline or the acid phosphatase of serum with the increase of alkaline phosphatase excreted in the urine. It seems reasonable to conclude that the nonsedimentable phosphatase is finely divided or soluble debris liberated from disintegrated tubular cells. On autolysis of cells phosphatase is liberated in association with material sedimentable at high speed. On further autolysis phosphatase is liberated in a nonsedimentable form.[8]

The relation of the presence of phosphatase-rich casts to the calcification which took place in the kidneys of the rats and to a lesser extent in those of the rabbits merits further study. Hepler, Simonds and Gurley [4] failed to find any relation between the presence of phosphatase and calcification. In our material calcification took place first in the cellular and granular casts rich in phosphatase. In some sections of the rat kidney calcification was so marked that it seemed to involve structures adjacent to the casts, extending even to glomeruli.

8. Kabat, E. A.: Science **93**: 43, 1941.

SUMMARY

In rats and rabbits, renal injury by uranium nitrate is followed by a conspicuous elevation of the concentration and total amount of alkaline phosphatase in the urine, associated with appearance of phosphatase-rich casts in the renal tubules. These casts are derived largely from the necrotized phosphatase-containing epithelium of the proximal convoluted tubules.

After the administration of uranium nitrate 13 to 46 per cent of the phosphatase in the urine excreted is contained in the urinary sediment. However, the concentration of this enzyme in the sediment is from six to sixty-five times higher than that in the supernatant fluid.

Recently regenerated renal tubular epithelium is poor in alkaline phosphatase.

FULMINATING MENINGOCOCCIC INFECTION

(THE WATERHOUSE-FRIEDERICHSEN SYNDROME)

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AND
WILLIS E. MANGES, M.D.
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In 1911 Waterhouse [1] reported an illness in a boy 8 months old which was characterized by initial drowsiness, vomiting, rapidly occurring cutaneous eruption, cyanosis and death within twelve hours of the onset. Necropsy disclosed massive bilateral adrenal hemorrhages. In the literature he found accounts of 15 other cases. In 1918 Friederichsen [2] described a similar illness in a boy 6 years old from whose blood he cultured a gram-negative diplococcus. Because of the high temperature and the rapidly fatal course in both his and the 27 cases which he reviewed, he believed that overwhelming infection was responsible for the disease. In these and other cases the symptoms, the signs and the course were so remarkably constant that subsequent authors have referred to the entity as the Waterhouse-Friederichsen syndrome.

Following Friederichsen's report, several reviews appeared in the medical literature. Thus Aegerter [3] collected 57 cases; Sacks,[4] 64 cases; Kunstadter,[5] 73 cases and Lindsay and others,[6] in 1941, 96 cases. Since 1941 29 additional cases [7] have been reported, bringing the total to 125. The rapid increase in the number of reports in recent years has brought about a general awareness of the entity and with this a reduction of the mortality, which previous to 1940 was 100 per cent. At the present time there are on record 5 cases [8] in which recovery followed prompt and vigorous treatment.

From the Clinical Laboratories, Jefferson Medical College Hospital.

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7. (*a*) Landis, J.: Schweiz. med. Wchnschr. **72**:179, 1942. (*b*) Holley, W. J.: Bull. Vancouver M. A. **18**:276, 1942. (*c*) Besser, R.: Rev. méd. de la Suisse Rom. **62**:569, 1942. (*d*) Gollick, W. A.: Psychiatric Quart. **16**:530, 1942. (*e*) Meningococcus Septicemia: Hemorrhage into Adrenal Glands, Cabot Case 26362, New England J. Med. **223**:383, 1940. (*f*) Banks, H. S., and McCartney, J. E.: Lancet **1**:219, 1942. (*g*) Leone, G. E.: Ann. Int. Med. **14**:2137, 1941. (*h*) Drummond, W. F., and Looke, T. B.: New Orleans M. & S. J. **94**:11, 1941. (*i*) Fox, S.: Brit. M. J. **2**:218, 1942. (*j*) Cunningham, J. A. K.: New Zealand M. J. **41**:238, 1942. (*k*) Hughs, J. F.: Brit. M. J. **2**:353, 1940. (*l*) Ross, E. S., and Schenkin, J. R.: New Orleans M. & S. J. **93**:294, 1940. (*m*) Monfort, J. A., and Mehrling, J. H.: Am. J. Dis. Child. **62**:144, 1941. (*n*) Michael, P., and Jacobus, L.: Arch. Pediat. **59**:141, 1942. (*o*) Kwedar, A. T.: Ann. Int. Med. **16**:787, 1942. (*p*) Carey, N. T.: ibid. **13**:1740, 1940. (*q*) Sharkey, J.: Lancet **2**:560, 1941. (*r*) Rucks, W. L., and Hobson, J. J.: J. Pediat. **22**:226, 1943. (*s*) Bickel, G.: Rev. méd. de la Suisse Rom. **60**:1058, 1940 (this article was unavailable to us). (*t*) Grace, W. H.; Harrison, C. V., and Davie, T. B.: Lancet **2**:102, 1940.

8. Carey.[7p] Sharkey.[7q] Rucks and Hobson.[7r] Bickel.[7s] Grace and others.[7t]

In most of the previous reports the clinical aspect of the syndrome has been discussed at length but comparatively little attention has been given the entity from the standpoint of pathology. We believe that the accompanying morphologic changes are of sufficient interest to warrant the reporting of 4 cases in detail.

REPORT OF CASES

CASE 1.—R. T., a white boy 8 months old, was admitted March 29, 1940 with a history of feverishness and perspiration for ten hours and dyspnea for four hours. The child was well developed, well nourished and acutely ill. The temperature was 105 F.; the respiratory rate was 60 and the pulse rate 160 per minute. A hemorrhagic rash, not present four hours previously, involved the skin of the entire body. Some of the areas had coalesced and measured as much as 0.5 cm. in diameter. They were dark red to purple and faded gradually into the surrounding skin. Externally they were smooth and blanched on direct pressure. The nasopharyngeal mucosa was congested. Moist rales were heard over the chest anteriorly. There were no signs of meningitis. The white blood cells numbered 6,100 per cubic millimeter. The cerebrospinal fluid showed 18 cells per cubic millimeter, no increase in globulin and no reduction of copper sulfate solution. With increase in dyspnea cyanosis appeared and the extremities became cold. The purpuric rash gradually progressed to areas of ecchymosis. The temperature, the pulse rate and the respiratory rate remained elevated, and death occurred fifteen hours after the onset of the first symptoms.

Necropsy disclosed a cutaneous eruption most pronounced over the lower part of the face, the neck, the extremities and the back. The lesions now measured as much as 2 cm. in diameter. The lungs, the liver and the kidneys were markedly congested. The spleen weighed 30 Gm. Its capsule was purplish gray and its pulp dark red and moderately firm. The thymus weighed 25 Gm. The lymph nodes were prominent. Dark red, almost purple adrenal glands were easily seen through the intact posterior peritoneum. Neither the size nor the shape of either gland was appreciably altered. The consistency was firm. Cut surfaces disclosed diffuse hemorrhagic extravasation throughout the medulla and cortex of each organ. Permission to open the calvarium was not granted. Culture of blood removed from the right side of the heart yielded a gram-negative bacillus identified as a member of the Friedländer group.

Microscopic examination of skin through a purpuric eruption disclosed diffuse engorgement of the dermal capillaries. Those immediately beneath the epidermis were relatively more prominent than the deeper ones. The walls of most of them were intact, but those of some were either partially or completely destroyed. Around the latter and throughout the immediate collagenous connective tissue there were large or small extravasations of blood. Where the capillary walls were indistinct or destroyed, polymorphonuclear leukocytes were found in considerable numbers both within their walls and in the immediate vicinity. A similar cellular infiltration was found around the hair shafts and the sweat glands. Hemorrhages here were less prominent. None of the vessels was thrombosed.

Sections of each adrenal gland disclosed diffuse infiltration by erythrocytes. The normal configuration of both the cortex and the medulla was still faintly discernible. Hemorrhages were most marked at the corticomedullary junctions, particularly in the zona reticularis, but they were also severe in the zona glomerulosa. The columns of the zona fasciculata were both widely separated and disrupted by erythrocytes. The cortical cells contained abundant, dark pink-staining, peripherally frayed cytoplasm and round vesicular nuclei. In some areas, where the columns were less distorted, there were small collections of polymorphonuclear leukocytes. Most of the medulla was replaced with either whole or laked blood. In focal areas, however, where medullary cells were still preserved and the hemorrhagic extravasation was less abundant, there was diffuse infiltration by polymorphonuclear leukocytes. This was particularly abundant about, and occasionally within, the walls of the larger thin-walled vessels. There were no thrombi. The periadrenal connective tissue showed marked edema and scattered foci of bacteria, polymorphonuclear leukocytes, plasma cells and lymphocytes. There were no hemorrhages.

The structure of the spleen was well preserved. The lymph follicles were prominent, sharply circumscribed and widely separated by a pulp somewhat less cellular than normal. In some areas the reticulum was prominent, but in others it was obscured. In the latter the outlines of the sinusoids were indistinct and the entire pulp moderately congested and diffusely sprinkled with increased numbers of polymorphonuclear leukocytes. Other cells which composed the pulp were lymphocytes, occasional plasma cells and scattered giant cells with one to three large dark nuclei and scanty cytoplasm. The vessels showed no thrombi or cellular

infiltration of their walls. The liver cords entirely lacked the normal radiating arrangement. The cells were swollen, and the cytoplasm was somewhat cloudy. There was slight diffuse fatty metamorphosis. The sinusoids were distended with erythrocytes, and the perisinusoidal spaces were filled with finely granular pink-staining material. There was diffuse infiltration by mature polymorphonuclear leukocytes, particularly marked in and about the sinusoids and around the portal radicles. Some of the central veins were crowded with similar cells. Scattered throughout, also, were round cells with scanty or abundant pink cytoplasm. The lungs showed congestion, edema and focal hemorrhages. Occasionally, polymorphonuclear leukocytes surrounded the septal capillaries. The kidneys showed diffuse congestion. The remaining organs, including the thymus and the lymph nodes, showed no histologic changes.

Sections of the skin, adrenal gland, liver, lung and bone marrow were stained for bacteria. Gram-negative bean-shaped diplococci morphologically similar to meningococci were present in the capillaries of the skin (fig. 1) and periadrenal connective tissue. None was found within either adrenal gland proper or in the other organs.

CASE 2.—R. M., a white girl 8 months old, was admitted April 11, 1943 with a history of a shaking chill of three minutes' duration thirteen hours previously. At that time two small red spots were noticed in the skin of the thorax and a third behind the right ear.

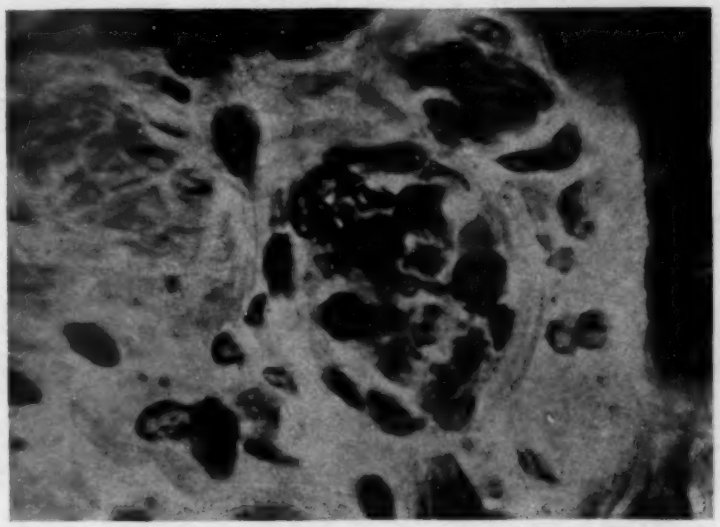

Fig. 1.—Photomicrograph showing many gram-negative bean-shaped diplococci within a dermal capillary. Two pair are in focus, while the rest are not. MacCallum-Goodpasture method; × 900.

Several hours later the rash was more generalized and the temperature was elevated. A physician who saw the child made a diagnosis of measles. Examination on admission disclosed an acutely ill, dyspneic, well nourished girl whose temperature was 104 F., pulse rate 110 and respiratory rate 60 per minute. Flat bluish red purpuric spots and areas of ecchymosis measuring as much as 3 cm. in diameter were present in the skin of the entire body. They were most prominent over the buttocks and genitalia. The lips and the fingers were cyanotic. Breath sounds were diminished over the lower portion of the right side of the thorax. There were no signs of meningitis. The white blood cells numbered 26,800 per cubic millimeter, with 29 per cent polymorphonuclear leukocytes and 68 per cent lymphocytes. In spite of sulfadiazine therapy, the condition of the patient rapidly deteriorated, and death ensued nineteen hours after the initial symptoms.

Necropsy disclosed a livid discoloration of the entire skin, most marked over the lower portion of the abdomen and the lower extremities. The dark bluish red purpuric spots and areas of ecchymosis were essentially unchanged. The adrenal glands were readily seen through the intact posterior peritoneum as dark bluish red masses. They were of approximately normal shape and size but somewhat increased in consistency. Each was diffusely hemorrhagic. The medulla showed some central disintegration but no liquefaction or clotting. The normal radiating structure of the cortices was obscured. Beneath the capsule of the

thymus, there were a few, scattered petechiae. The thymus was otherwise normal. The lungs showed congestion, edema and in their posterior portions patchy areas of atelectasis. The pia-arachnoid vessels were markedly congested, but an exudate was not detected. The rest of the organs, including the spleen and the liver, revealed no gross changes.

A postmortem smear of the spinal fluid disclosed no organisms, and a culture was sterile after ninety-six hours of incubation. Culture of blood obtained from the right side of the heart yielded a growth of Staphylococcus aureus and an occasional colony of Streptococcus haemolyticus.

Sections of skin through a purpuric area disclosed marked congestion of the dermal capillaries. These were particularly prominent immediately beneath the epidermis. Most of the capillaries showed nothing more. A few, however, revealed prominent endothelial cells and an infiltration of their walls and pericapillary tissue by polymorphonuclear leukocytes and occasional lymphocytes and plasma cells. Several of these capillaries were partially or completely occluded with hyaline thrombi and nuclear fragments (fig. 2). There was moderate

Fig. 2.—Photomicrograph showing several dermal capillaries completely occluded with thrombi of hyaline material and nuclear fragments. Their walls and the surrounding connective tissue contain extravasated erythrocytes and a few polymorphonuclear leukocytes, lymphocytes and plasma cells. Hematoxylin and eosin; × 400.

erythrocytic extravasation into the surrounding connective tissue. The capillaries about the hair shafts were moderately congested. They showed no hemorrhages or inflammation.

Each adrenal gland disclosed a severe diffuse hemorrhage that was slightly more prominent at the junction of the cortex and the medulla and in the zona reticularis. The normal structure was completely distorted, although radial striations of cortical cells were still faintly discernible. Hemorrhage was so severe that there were no remaining strands of normal tissue as in the previous case. Instead the cells were single and in groups of not more than three or four. Their cytoplasm was pink, peripherally frayed and abundant. Their nuclei were essentially normal. The medulla was completely replaced with hemorrhage. Infiltrations of polymorphonuclear leukocytes were entirely absent. The periadrenal connective tissue was edematous, and the capillaries were congested. The endothelial cells of some of the capillaries were prominent. Both the capillaries and the surrounding connective tissue were lightly infiltrated by polymorphonuclear leukocytes. The latter also contained scattered lymphocytes. Thrombi were not found in any of the vessels.

The follicles of the spleen were prominent and surrounded with broad bands of erythrocytes. The reticulum in these hemorrhagic areas was either barely visible or entirely absent.

There was only an occasional polymorphonuclear leukocyte. The remaining pulp consisted of an abundant amount of reticulum, prominent, distended and empty sinusoids and fewer erythrocytes. There were present also some round and plasma cells and occasional giant cells with one to three nuclei and scanty cytoplasm. Sections of the liver were identical with those of the former case. The liver cords were irregularly arranged. The cytoplasm of their cells was somewhat swollen, cloudy and contained many fat globules. The sinusoids were markedly congested and the perisinusoidal spaces filled with pink-staining granular material. There was a diffuse sprinkling of mature polymorphonuclear leukocytes. In addition, some of the sinusoids and practically all of the portal radicles contained round cells with scanty or abundant pink cytoplasm and large, round, evenly staining nuclei. There were no thrombi. The capillaries of the thymus were congested. There were, in addition, foci of subcapsular and intracapsular erythrocytic extravasation but no other abnormalities. The lungs showed congestion, edema and partial atelectasis. The remaining thoracic and abdominal organs showed no histologic changes. The meninges of the brain were diffusely and heavily infiltrated by leukocytes, among which polymorphonuclear cells predominated. The vessels were engorged. The perivascular spaces were dilated and occasionally contained erythrocytes. Nissl's stain disclosed peripheral dispersion of the Nissl substance of the ganglion cells with swelling of the cells and fragmentation.

Sections of skin, adrenal gland, lung, liver, spleen, intestine and bone marrow were stained for bacteria. Numerous gram-negative bean-shaped intracellular and extracellular diplococci morphologically similar to meningococci were found in the thrombosed vessels of the skin and in the immediately surrounding connective tissue. None was found in other organs.

Case 3.—E. P., a white youth 18 years old, died in the accident ward April 26, 1935. A history is not available, but from the autopsy findings and the fact that he died before he could be transferred to the hospital we assume that his illness was similar to that in the other cases here described.

Necropsy disclosed a well developed, well nourished white boy. There was marked purplish red and livid discoloration of the skin of the entire body, most prominent in the face and neck. Hemorrhagic extravasations were present in each conjunctiva. The mucous membranes of the lips and mouth were cyanotic. The adrenal glands maintained approximately their normal shape and size but were rather soft. They were uniformly dark red. Cut surfaces disclosed diffuse hemorrhages throughout the cortex and medulla. Beneath the endocardium of the left ventricle, there was a "rather large" superficial hemorrhage. The spleen weighed 260 Gm. It was soft, the pulp was dark red and the follicles were prominent. The liver weighed 1,970 Gm. It was very soft, dark reddish brown and friable. The abdominal lymph nodes measured not more than 1 cm. in diameter. The thymus is not mentioned in the protocol and so was apparently normal. Beneath the periosteum of the calvarium there were numerous small dark red extravasations of blood. The vessels of the pia-arachnoid over the convexity of the brain were markedly congested but there was no exudate. The aural cavities and the sinuses showed no infection. Spinal fluid removed at autopsy disclosed a dextrose level of 45 mg. per hundred cubic centimeters and 4 cells per cubic millimeter. Culture of both spinal fluid and cardiac blood yielded pure growths of the meningococcus.

Sections of the adrenal glands showed diffuse congestion and hemorrhages. The latter were most marked at the junction of the cortex and the medulla and in the zona reticularis. In some areas the entire structure was erased and the cells destroyed. In others, where the hemorrhages were less severe, the medulla was essentially normal, while the cortical cells of the zona fasciculata were merely separated by greatly congested sinusoids. The corresponding zona glomerulosa showed little congestion and no distortion of its cells. Nowhere was there infiltration by polymorphonuclear leukocytes. The vessels contained no thrombi. The periadrenal connective tissue showed edema and some erythrocytic extravasation. The vessels were congested. There was no inflammation. Bacteria were not found in sections stained with hematoxylin and eosin. Tissue was not available for special staining.

Sections of the spleen were essentially similar to those in case 1. The follicles were prominent and widely separated with pulp. Although the reticulum was diffusely increased, the sinusoids were not conspicuous. In some areas erythrocytes were so abundant as to overshadow other elements. Polymorphonuclear leukocytes were increased but were less prominent than in case 1. Giant cells were only occasionally present. Sections of the liver were similar to those found in cases 1 and 2 except that the normal radiating arrangement of the liver cords was better preserved. The liver cells were swollen and cloudy. There was no fatty change. The sinusoids were markedly congested, and the perisinusoidal spaces were filled with granular pink-staining material. The entire parenchyma, particularly in and about the sinusoids, was diffusely infiltrated by mature polymorphonuclear leukocytes. Other cells were not present except in the portal radicles, where there were collections of lymphocytes

and scattered plasma cells. Immediately beneath the endocardium of the left ventricle were several sharply circumscribed hemorrhages composed of both normal and laked erythrocytes. The capillaries throughout were congested, and many contained increased numbers of polymorphonuclear leukocytes. Occasionally their walls and the immediately adjacent connective tissue were permeated with similar cells. Small collections of polymorphonuclear leukocytes dissociated from blood vessels were also seen between the muscle fibers and in the supporting connective tissue. There were no areas of necrosis, thrombi or bacteria seen in the sections stained with hematoxylin and eosin. The lungs showed congestion and edema. Within the septums and in some of the alveoli there were isolated and focally collected bean-shaped diplococci morphologically similar to meningococci. Tissue for bacterial stains was not available. Sections of the brain disclosed marked congestion of the pia-arachnoid vessels but no inflammation. Sections of the remaining organs were essentially normal.

CASE 4.—T. D., a white boy 14 months old, was admitted April 14, 1943 with a history of running nose and slight cough for two days. Eleven hours previously he vomited milk, was restless and cried aloud. Two hours later his mother noticed that he was "stiff all over" and immediately afterward he underwent a convulsion. The latter recurred at about thirty minute intervals, each lasting twenty minutes, until admission nine hours later. The boy was well developed and well nourished but apathetic. Petechiae 2 mm. in diameter were present in the skin of the chest, back and extremities. The skin was warm and dry. There were occasional clonic contractions of the right arm and leg, but there was no rigidity of the neck. Crusted serosanguineous material was present about the external nares. Rales were heard over the apexes of both lungs and over the base of the right lung. The white blood cells numbered 3,200 per cubic millimeter, 43 per cent being polymorphonuclear leukocytes. The spinal fluid contained 960 cells per cubic millimeter, 55 per cent being polymorphonuclear leukocytes, and many gram-negative bean-shaped intracellular and extracellular diplococci. A pure culture of meningococcus type I was obtained from the fluid. Similar organisms were grown from the nasopharynx. A blood culture was sterile at the end of forty-eight hours' incubation. In spite of treatment with sodium sulfadiazine, the temperature gradually rose to 105.4 F. and the pulse rate to 140 and the respiratory rate to 60 per minute. The child died twenty-three hours after the initial vomiting.

At necropsy the skin disclosed scattered pinpoint petechiae together with generalized irregular ill defined "blotchy" areas of livid discoloration. There were no frank hematomas. The lungs were congested and contained a few bright red hemorrhages measuring as much as 0.3 cm. in diameter. Posteriorly there were small patchy areas of atelectasis. The thymus weighed 15 Gm. The lymph nodes were prominent. The adrenal glands were of approximately normal shape, size and consistency. The cortex and medulla of the right adrenal gland in its midportion disclosed a dark red hemorrhage 0.6 cm. in diameter. The medulla of the left adrenal gland was slightly congested. The spleen weighed 25 Gm. It was firm and irregularly congested. The liver was grossly normal, weighing 310 Gm. The brain weighed 940 Gm. Its cerebral hemispheres were enlarged and showed considerable flattening of the convolutions. The vessels of the pia-arachnoid were congested, but the meninges were grossly free of exudate.

Sections from the purpuric foci in the skin disclosed marked erythrocytic engorgement of the dermal capillaries, especially of those immediately beneath the epidermis. Surrounding the vessels were occasional plasma cells and lymphocytes. There was no erythrocytic extravasation or capillary thrombosis.

Sections of the left adrenal gland showed a well preserved cortex. The central portion of the gland contained only small islands of medullary cells. The remainder consisted of loose pink-staining fibrillary connective tissue diffusely permeated with small and large, thin-walled capillaries (fig. 3). In many areas these were widely patent and empty. In others they were so engorged with erythrocytes that the supporting connective tissue was barely visible. Several small foci of diffuse erythrocytic extravasation were limited to the medulla. Where the medullary cells were abundant, the capillaries, although congested, were relatively inconspicuous. Sections of the right adrenal gland at the level of the hemorrhage disclosed a sharp line of demarcation between morphologically normal and abnormal adrenal tissue. In the latter, only a bare outline of the former structure remained, most of the cells having been completely replaced with erythrocytes. The corticomedullary junction was represented by a collagenous band of fibrous tissue. As in the other cases, erythrocytes were most abundant at the junction of the cortex and the medulla and in the zona glomerulosa. There were no polymorphonuclear leukocytes, thrombi or bacteria. The medulla of the normal portion immediately adjacent to the hemorrhage was broad, cellular and somewhat avascular. Elsewhere, as in the left adrenal gland, there were focal areas of hypoplasia in which the capillaries were either empty or congested. In some portions the loose fibrillary supporting

connective tissue was filled with erythrocytes. The periadrenal connective tissue of each organ was edematous, and the capillaries were congested.

The lymph follicles of the spleen were conspicuous and were ringed with broad collars of erythrocytes as in case 2. In some areas the pulp showed a prominent pink-staining broad reticulum whose sinusoids were widely patent and either empty or filled with blood. In other areas the reticulum was overshadowed with erythrocytes. Polymorphonuclear leukocytes were occasionally seen, but giant cells were not present. The liver cords lacked the normal radiating pattern. There were congestion of the sinusoids and slight edema of the perisinusoidal spaces but nowhere, including the portal areas, were there cells of inflammatory origin. The thymus revealed no histologic changes other than a few, scattered collections of eosinophils. The lungs showed congestion and edema. In the peribronchial connective tissue there were lymphocytes, a few plasma cells and occasional polymorphonuclear leukocytes. Other organs, including the lymph nodes, showed only congestion. Sections of the brain disclosed diffuse infiltration of

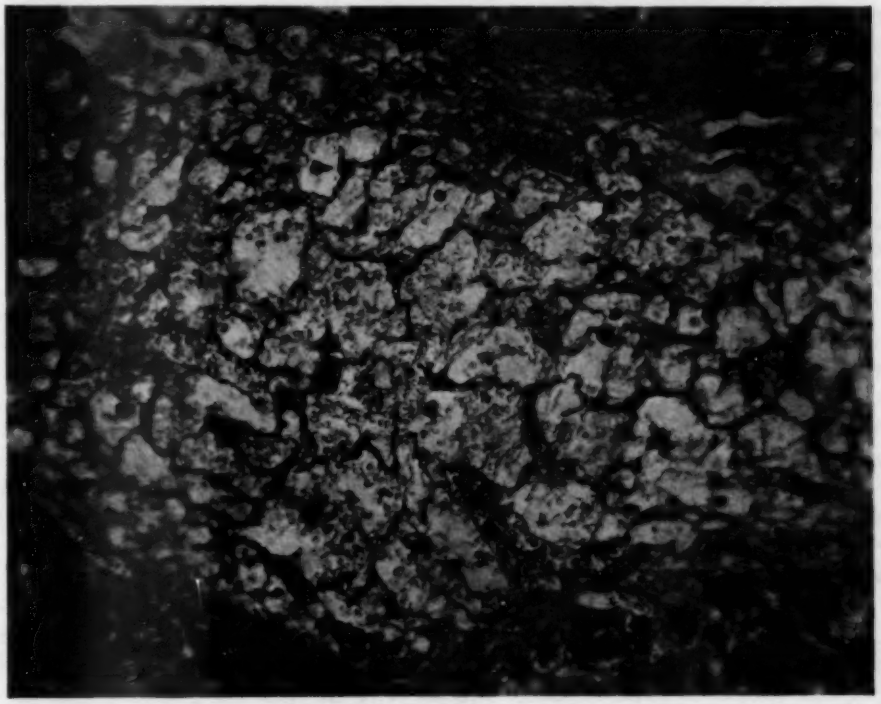

Fig. 3.—Photomicrograph showing an area of hypoplasia of the medulla of the left adrenal gland (case 4). It consists of loose fibrillary connective tissue in which there is engorgement of the capillaries with beginning extravasation of erythrocytes. Note the absence of medullary cells. The inner portion of the zona reticularis of the cortex is seen at the periphery. Hematoxylin and eosin; × 200.

the meninges by polymorphonuclear leukocytes. The capillaries were congested. The ganglion cells of the cortex showed marked toxic changes.

Bacterial stains of sections of the skin showed many gram-negative bean-shaped diplococci morphologically similar to meningococci within the capillaries of the corium. They were not present in sections of the adrenal glands, liver, spleen, lymph nodes or lungs.

COMMENT

Because of the rapid onset of symptoms, the high fever, often associated with leukocytosis and the fulminating course with fatal termination, the syndrome has long been regarded as that of overwhelming septicemia. Although meningococci have been shown to be the causative agent in over 60 per cent of the cases,[4]

we believe that this figure is much too low and that most, if not all, of the patients from whom other organisms were isolated were nevertheless infected with meningococci. In support of this statement we refer to cases 1 and 2 in which a member of the Friedländer group and Staph. aureus, respectively, were isolated from the blood post mortem, and yet bacterial stains of sections of the skin and the adrenal glands in case 1 and of the skin in case 2 disclosed gram-negative bean-shaped diplococci morphologically similar to meningococci.

Clinically an etiologic diagnosis may be established in one of several ways. Perhaps the simplest, and surely the quickest, is to make a smear directly from a purpuric eruption and stain for bacteria. McLean and Caffey [9] found meningococci in such smears in 83 per cent of cases examined. In fact, according to them, this method was even more reliable than culturing the blood. In 16 cases in which both blood cultures and cutaneous smears were simultaneously examined, the former were positive for meningococci in 10, while the latter were positive in 13. Occasionally, gram-negative intracellular diplococci may be found within polymorphonuclear leukocytes in an ordinary blood smear.[10] They may also be isolated from the nasopharynx as in case 4 here reported. Finally, since meningitis is associated with the septicemia in a small proportion of cases, the meningococci may be recovered from the spinal fluid by direct smear or culture or both. At necropsy meningococci have been repeatedly isolated from the cardiac blood and occasionally from the spinal fluid. In addition, they have been found in sections of adrenal glands,[11] skin,[7e] spleen,[7b,t] and renal glomeruli, lungs and liver.[7t]

While simple engorgement of the dermal capillaries [12] is frequently the only histologic alteration in the skin, associated hemorrhages [13] have also been observed. On the other hand, capillary thrombi [7l] have been recorded only rarely. They were present in our case 2. The belief that the lesions of the skin are the result of direct involvement of the capillaries and arteries by the causative organisms [14] is given additional support by the finding of meningococci within such vessels in all of our cases in which sections of the skin were stained for bacteria (cases 1, 2 and 4). Organisms were particularly abundant in case 2, being found not only within the thrombi but also in the walls of the vessels and in the surrounding connective tissue.

Since gram-negative diplococci had been previously reported in the adrenal glands [11] and were found also in the periadrenal capillaries in our case 1, it is probable that the hemorrhages here, as in the skin, are caused by the action of the organisms directly on the capillary walls. Why the adrenal glands should be so regularly involved, however, while hemorrhages in other serosal surfaces and organs are so inconstant is not at all clear. Of the various theories advanced, there are only two which seem plausible. In the infant the adrenal medulla while undergoing involution has a rich supply of capillaries and therefore readily lends itself to trauma by a variety of agents.[15] In support of such involutionary changes Michael and Jacobus [7n] found necrosis of the medulla and zona reticularis in their first case. In our case 4 there was a single small hemorrhage in the right adrenal gland. Both adrenal glands showed only scattered islands of intact medullary cells. The remainder of the medullary region consisted of loose fibrillary connective tissue in

9. McLean, S., and Caffey, J.: Am. J. Dis. Child. **42**:1053, 1931.
10 Andrewes F. W.: Lancet **1**:1116, 1940. Kwedar.[7o]
11. Kunstadter.[5] Kwedar.[7o]
12. (*a*) Levinson, S. A.: J. Pediat. **14**:506, 1939. (*b*) Kwedar.[7o]
13. Friederichsen.[2] Monfort and Mehrling.[7m]
14. (*a*) Rabinowitz M. A.: Am. J. M. Sc. **166**:513, 1923. (*b*) Monfort and Mehrling.[7m]
15. Firor, W. M.: South. M. J. **30**:306, 1937. Kunstadter.[5] Levinson.[12a]

which there were many thin-walled engorged capillaries. In the left adrenal gland the medulla, which grossly showed no hemorrhage, had capillaries so dilated that the intervening connective tissue was barely visible. In a few areas the capillary boundaries were broken and erythrocytes permeated the surrounding structures. Without stretching one's imagination too far, one can readily see how an increase in extravasated erythrocytes would produce the massive hemorrhages which are so characteristically found in the syndrome. While such involutionary changes are not present in adults, there still remains some spontaneous affinity of the capillaries for even minor traumas.[16] The second theory to appear plausible is that of the selective action of meningococci for structures of ectodermal origin, first voiced by Maclagan and Cooke [17] and subsequently supported by Rabinowitz,[14a] Kunstadter [5] and others.

The microscopic changes in the liver have rarely been described. Levinson [12a] stated that eosinophils, lymphocytes, plasma cells and a few polymorphonuclear leukocytes had been found beneath Glisson's capsule and considered by some authors as evidence of anaphylaxis. In the first 3 cases of the present report the distortion of the normal radiating structure, the swelling of the liver cells, the sinusoidal congestion, the perisinusoidal edema and the diffuse infiltration by polymorphonuclear leukocytes were so constant and so similar as to suggest that they are part of the syndrome. They are undoubtedly manifestations of septicemia. It might be argued that the infiltrations represent extramedullary hemopoiesis. While this cannot be absolutely denied in regard to the patients in cases 1 and 2, who were each 8 months old, we should nevertheless emphasize that the cells were mostly mature polymorphonuclear leukocytes. Certainly the diffuse cellular infiltration of the liver of the patient in case 3, who was 18 years old, cannot be ascribed to hemopoiesis. The absence of inflammation in the liver of the fourth patient can be explained by the fact that in this child meningitis was the predominating lesion while the generalized systemic disturbance was secondary.

Because of the enlargement of the thymus and the prominence of the lymph nodes, of Peyer's patches and of the follicles of the spleen, Rabinowitz [14a] linked the syndrome with status thymicolymphaticus. Subsequently several authors [18] subscribed to his observation. Without delving into a discussion of this highly controversial subject, we wish to point out that in none of our cases was the thymus or the lymphatic system more "hyperplastic" than one would expect in any previously healthy and well nourished child who has been ill only a few hours or at the most a few days before death. All reports without exception state that the patients were previously in perfect health.

Although it is the consensus of most authors who have described the syndrome that adrenal apoplexy is responsible for the death of the patients, we concur with Aegerter [3] in his statement that "there is no proof that death in the Waterhouse-Friederichsen syndrome is due to adrenal failure rather than to toxicity caused by invasion of the blood stream." John H. Musser in commenting on the cases presented by Ross and Schenkin [71] said that he believed the hemorrhage in the adrenal glands was coincidental. To support this contention we refer to our case 4. With the exception that the symptoms and signs were more meningeal than systemic and that the purpuric spots were not as large as in the other cases, the course of the disease was indistinguishable from that in cases 1 and 2. There was only a single small circumscribed hemorrhage in the right adrenal gland. Furthermore, McLean

16. Thorstad, M. J.: Am. J. Surg. **55**:44, 1942.

17. Maclagan, P. W., and Cooke, W. E.: Lancet **2**:1054, 1916.

18. Sacks.[4] Lindsay and others.[6] Monfort and Mehrling.[7m] Michael and Jacobus.[7n] McLean and Caffey.[9]

and Caffey [9] reported autopsies on 7 patients with meningococcic bacteremia all of whom had purpuric eruptions and whose illnesses clinically were more or less identical. Three showed adrenal hemorrhages while 4 did not. The average duration of the illness in the former group was twenty-eight hours. In the latter it was 38 hours. Finally, Lindsay and others [6] stated that in 95 per cent of the cases the adrenal hemorrhages are bilateral and that these vary from petechiae to massive extravasations. In those in which only petechiae are found and in others in which the hemorrhage is unilateral there is no reason to suppose that adrenal insufficiency exists. Yet in all these cases the rapidly fatal course with terminal manifestations of shock are characteristically present.

SUMMARY

Four cases of the Waterhouse-Friederichsen syndrome are presented, bringing the total number recorded to 125. In 3 cases the adrenal hemorrhages were bilateral and massive. In the fourth case only a single small circumscribed hemorrhage was found in the right adrenal gland, while both medullas showed areas of hypoplasia. Sections of skin were examined in 3 cases and showed respectively: congestion of the dermal capillaries; congestion, hemorrhages and inflammation, and congestion, hemorrhages, inflammation and thrombosis. In 3 cases in which the symptoms were predominately septicemic there were dissociation of the liver cords, cloudy swelling of their cells, sinusoidal congestion, perisinusoidal edema and slight diffuse infiltration by polymorphonuclear leukocytes. Meningococci were isolated in pure culture from the spinal fluid and the cardiac blood post mortem in 1 case and from the spinal fluid and the nasopharynx ante mortem in 1 case. Postmortem blood cultures in the remaining 2 cases yielded respectively a member of the Friedländer group and Staph. aureus. Sections of the skin in each of these and of the adrenal gland in the former disclosed within the capillaries gram-negative bean-shaped diplococci morphologically similar to meningococci. Because of these findings it is thought that most, if not all, of the cases of the Waterhouse-Friederichsen syndrome in which other organisms have been isolated were nevertheless caused by meningococci.

Case Reports

EMBOLISM FROM LIVER TISSUE IN PULMONARY ARTERY

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MEDICAL CORPS, ARMY OF THE UNITED STATES

Considering the number of deaths following accidental trauma to abdominal viscera, rarely can it be demonstrated that the death was due to extensive pulmonary embolism. Death occurred within forty-five minutes from an unusual form of embolism in a soldier unavoidably run over during night field maneuvers.

Embolus of liver tissue in the pulmonary artery: *A*, pulmonary valve; *B*, pulmonary artery; *C*, hepatic tissue; *D*, right ventricle.

The only bones fractured were the right sixth and seventh ribs posteriorly and yet the severity of compression was sufficient to rupture the liver and to drive a segment of it into the inferior vena cava and on into the pulmonary artery (figure). This fact was established by locating the rent in the inferior vena cava and the macerated hepatic tissue in both the right auricle and the pulmonary artery. In the latter the lumen was completely occluded for a distance of 5 cm. beginning 2 cm. from the valvular orifice. No other significant pathologic occurrence was observed.

EXTRAMAMMARY PAGET'S DISEASE

LAWRENCE PARSONS, M.D., AND HAROLD E. LOHLEIN, M.D., RENO, NEV.

Extramammary Paget's disease has been rarely reported. In an excellent review Weiner,[1] who collected 57 cases reported as instances of extramammary Paget's disease and reported 1 additional case, finally settled on 15 (including his own) as being true instances of this rare condition. In 4 of those the disease involved the male genitals; in 8, the vulva, and in 3, the axilla.

There seems to be a moot question as to the exact number of cases of true extramammary Paget's disease which have been reported. Weiner's [1] estimate of a total of 15, including his own, is in our opinion approximately accurate. Pinkus and Gould,[2] in a more recent review of the subject, excluded a number of the cases which Weiner accepted and reduced the total number of true cases to 9. However, they included among these 9 several which in our opinion Weiner justifiably rejected. Consequently we feel that the last word has not yet been said regarding the total number of acceptable cases. The true number would seem to be small.

We believe that the term "extramammary Paget's disease" should be restricted to those cases in which the lesion occurs apart from the breast and its covering skin and is from the standpoint of histology entirely similar to the classic lesion in the breast. That is, the epidermal changes should be essentially identical with those observed in Paget's disease of the nipple and areola and, further, carcinoma of the underlying sweat glands (usually the apocrine) should be present. However strikingly similar both clinically and pathologically Bowen's dermatosis in certain cases listed by Pinkus and Gould,[2] the syphilitic erythroplasia of Queyrat (Pinkus and Gould [2]), melanoma with intradermal spread as described by Stout [3] and even the carcinoma of the rectum with extension to the skin reported by Pinkus and Gould [2] may be to Paget's disease, we feel that these conditions should not be considered to be true extramammary Paget's disease.

Stout's statement [3] seems to us to epitomize the point of view concerning true extramammary Paget's disease:

> After studying all of the case reports analyzed by Weiner and a few more which he failed to include, the writer is persuaded that his conclusions are well taken and that he has settled the question: Extramammary Paget's disease does occur, and it consists of peculiar skin lesions which are clinically and microscopically similar to Paget's disease of the nipple, are associated usually with an underlying apocrine sweat gland carcinoma, and are quite possibly secondary to it.

Since Weiner's [1] review we have been able to find but 2 cases reported as instances of extramammary Paget's disease in a review of the literature. The first, reported by Hagedoorn,[4] was that of a woman aged 56 who had been treated for trachoma since childhood and who had both carcinoma of the conjunctiva of an eyelid and carcinomatous changes in the meibomian glands which simulated Paget's disease. The photomicrographs are not convincing and we doubt that it is a true case of extramammary Paget's disease. The second, reported by Komori,[5] was that of a 9 year old girl who had an itching lesion of the skin on the mons pubis and labia which had been present for five years. The photomicrograph is quite suggestive of Paget's disease but both the author's description of the microscopic changes (English abstract) and a translation of the entire article leave considerable doubt as to the true nature of the lesion.

1. Weiner, H. A.: Am. J. Cancer **31**:373, 1937.
2. Pinkus, H., and Gould, S. E.: Arch. Dermat. & Syph. **39**:479, 1939.
3. Stout, A. P.: Am. J. Cancer **33**:196, 1938.
4. Hagedoorn, A.: Brit. J. Ophth. **21**:234, 1937.
5. Komori, S.: Mitt. a. d. med. Akad. zu Kioto **24**:42, 1938.

REPORT OF A CASE

S. L. W., a white man 59 years old (in 1943) was first seen by one of us (H. E. L.) in November 1940. He complained of an itching cutaneous lesion involving the right groin and part of the right side of the scrotum which began in the fall of 1937 as a small red papule. He was a robust man in apparent good health, and the examination disclosed nothing abnormal except the lesion of the skin, occupying an area about 8 cm. by 6 cm. in size, involving the right groin and a small portion of the skin of the scrotum adjacent to it. For several centimeters beyond the main area the skin presented a peculiar brownish discoloration. The lesion was sharply demarcated from the surrounding skin and was elevated about 1.5 cm. above the level of the skin. It was pink and had a granular, verrucous appearance. The

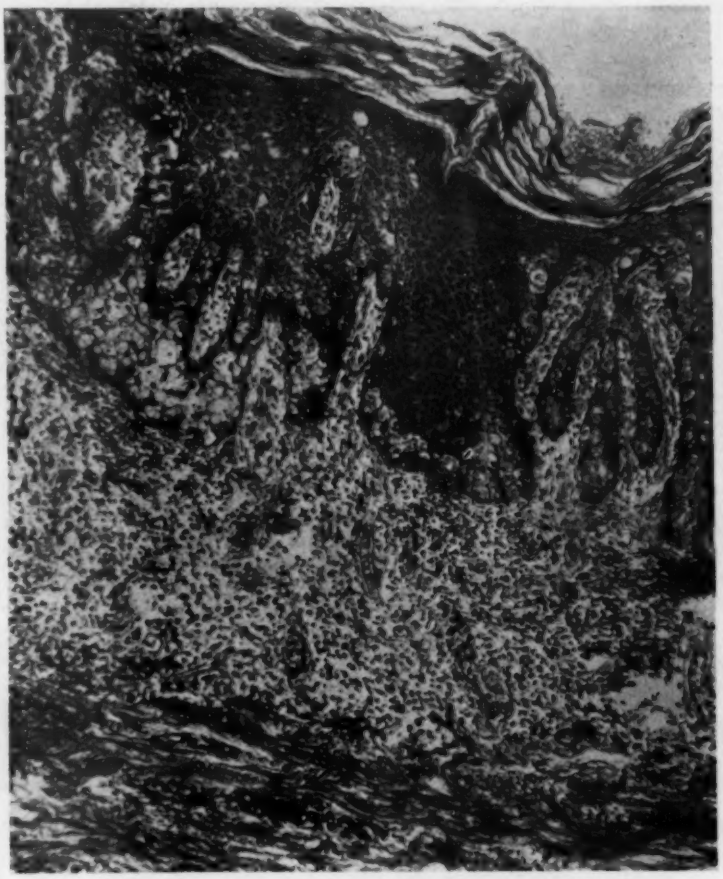

Fig. 1.—Paget's lesion in the epidermis; × 150.

surface was moist with a watery secretion and bled freely when scraped with a scalpel. It was quite insensitive, and the scraping was appreciated by the patient since the area itched a good deal. There were no palpable inguinal lymph nodes.

The tentative diagnosis was venereal granuloma but no Donovan bodies were found on repeated examination. The Wassermann and Kahn tests of the blood were negative. The Frei test for lymphogranuloma venereum in January 1941 was negative.

A biopsy was done on Feb. 3, 1941, and the patient was admitted to St. Mary's Hospital February 23. Kolmer, Wassermann and Kahn tests of the blood serum were negative. The urine was normal. The blood showed a normal hemoglobin content, and the red cell count was normal. The leukocytes numbered 15,500 per cubic millimeter, with 25 per cent lymphocytes, 3 per cent monocytes, 5 per cent eosinophils and 67 per cent neutrophils; of the latter 3 were nonfilamented forms.

Complete surgical excision of the growth was done February 24. The inguinal lymph nodes were not removed. The postoperative course was uneventful, and the patient was discharged from the hospital March 20. The wound healed slowly. Roentgen treatments were given, but the growth gradually recurred, and on Jan. 9, 1942 a second excision was done. At the time of writing, May 25, 1943, the lesion has reached its former proportions as well as having spread across the pubis to the opposite groin. There are still no palpable inguinal lymph nodes.

The specimen removed for biopsy Feb. 3, 1941 was 2.5 cm. in size, and the epidermal surface was verrucous in appearance. The cut surface showed a fairly sharp line of demarcation between the edematous corium and the cauliflower-like epidermal growth. Microscopically, the epidermis was markedly thickened, with considerable hyperkeratosis. The rete pegs were markedly elongated and widened. The papillae of the corium were heavily infiltrated with plasma cells together with a few lymphocytes and eosinophils. No Donovan bodies were

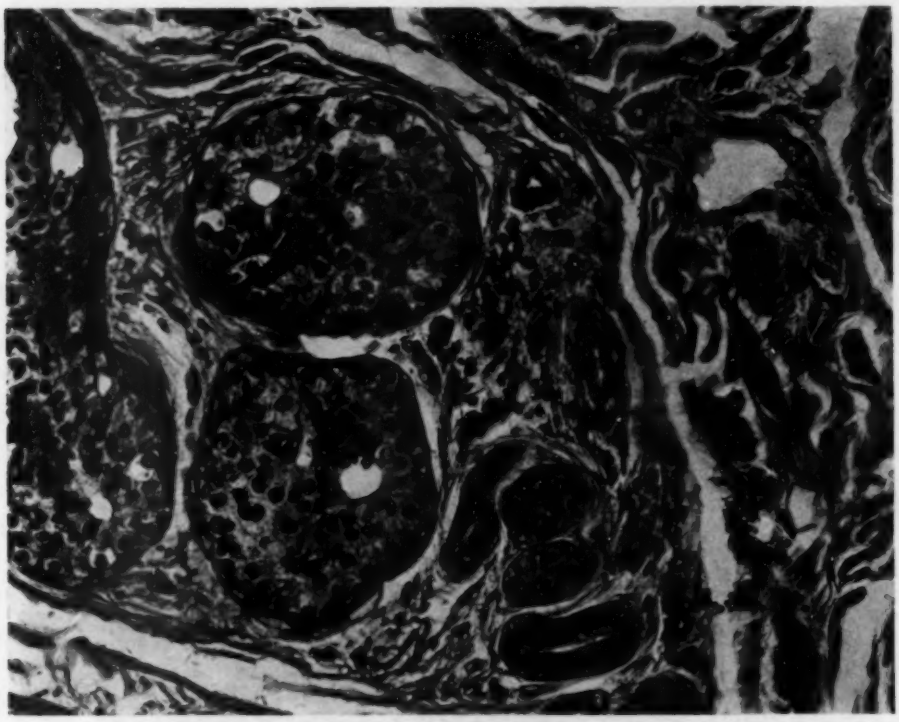

Fig. 2.—Pagetoid proliferation in a sweat gland; × 200.

found. The epidermis was markedly edematous, and numerous hydropic cells identical with those seen in Paget's disease of the nipple were seen. Many mitoses were present throughout the epidermal cell layer. The lesion was identical with Paget's disease of the nipple.

The lesion removed by surgical excision February 24 showed the same epidermal changes as the biopsy and, in addition, pagetoid proliferation of the sweat glands, apparently the eccrine. The cells lining the excretory sweat glands had enlarged hyperchromatic nuclei with hydropic changes in the cytoplasm, and occasional mitoses were seen.

The tissue removed Jan. 9, 1942 showed changes entirely similar to those found in February 1941; both the epidermis and the sweat glands showed involvement typical of Paget's disease and indistinguishable from that in a case of Paget's disease involving the female breast.

Figure 1 shows the Paget's lesion in the epidermis under medium power, and figure 2 is a high power view of a few of the sweat glands with pagetoid proliferation. Several normal sweat glands, apparently of the secretory (eccrine) type, are seen to the right of the carcinomatous glands.

COMMENT

It is not within the scope of this report to enter into a full discussion of the disputed nature of Paget's disease of the nipple. It is the opinion of Muir [6] that the epidermal changes characteristic of Paget's disease of the nipple are due to intraepithelial spread of cancer cells from an underlying carcinoma of the mammary parenchyma or of ducts of the nipple. The description and illustrations of his cases are convincing indeed, and his views may eventually be accepted. Practically every breast with Paget's disease will, if examined with sufficient care, including the ducts of the nipple, show duct or intraductal carcinoma. It is with Muir's work in mind that we feel that the term "extramammary Paget's disease" should be restricted by definition to include only those cases presenting both the classic epidermal changes and underlying carcinoma of the sudoriferous glands. We must admit, however, that the theory of two independent types of cancer, that of the epidermis with Paget's cells and that occurring in the glandular epithelium beneath it, although in our opinion unproved, is attractive on the basis of the possibility of some carcinogenic influence being exerted simultaneously on both the epidermis and the underlying glands.

SUMMARY

A case of extramammary Paget's disease involving the skin and the sweat glands of the groin and scrotum is reported. This is approximately the sixteenth case of this rare condition which has been reported.

6. Muir, R.: J. Path. & Bact. 49:299, 1939.

PRIMARY INFRAPAPILLARY ADENOCARCINOMA OF THE DUODENUM

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The case of primary infrapapillary adenocarcinoma of the duodenum to be reported here is of interest because of its rarity and because of the associated lesions and freedom from clinical symptoms and signs.

A. B., a woman aged 70, was referred to the Bronx Hospital by Dr. Harry Lipkin Feb. 17, 1943 because of an acute cerebral accident which resulted in left-sided hemiplegia. A similar episode associated with temporary facial palsy had occurred approximately three weeks previously. There was an antecedent history of hypertension with one attack of dizziness three months before the present episode. The typical neurologic signs of a lesion in the right internal capsule, probably a cerebral hemorrhage, were present. The blood pressure was 210 mm. systolic and 90 mm. diastolic. There was a murmur over the mitral area. The patient died on the second day of hospitalization. Permission for necropsy included only examination through an abdominal incision.

Just beyond the pyloric ring there were two duodenal ulcers, each approximately 0.5 cm. in diameter. At approximately the duodenojejunal junction, on the posterior wall there was an irregularly rounded sessile tumor, 6 by 6 cm., with elevated, rolled margin. Some superficial ulceration appeared to be present in the slightly depressed central portion where the tumor infiltrated the wall as far as the serosa. The colon was the seat of several small, scattered adenomatous polyps, each approximately 0.5 cm. in diameter, and a submucous lipoma 2 cm. in diameter. Each kidney measured 10 by 5 cm. and weighed 150 Gm. The left kidney presented a moderate degree of hydronephrosis. The larger blood vessels revealed considerable atherosclerosis, the coronary arteries being calcified but not occluded. Calcific changes were also present in the mitral and aortic valve cusps, which were quite rigid. The lungs were the seat of marked edema and congestion.

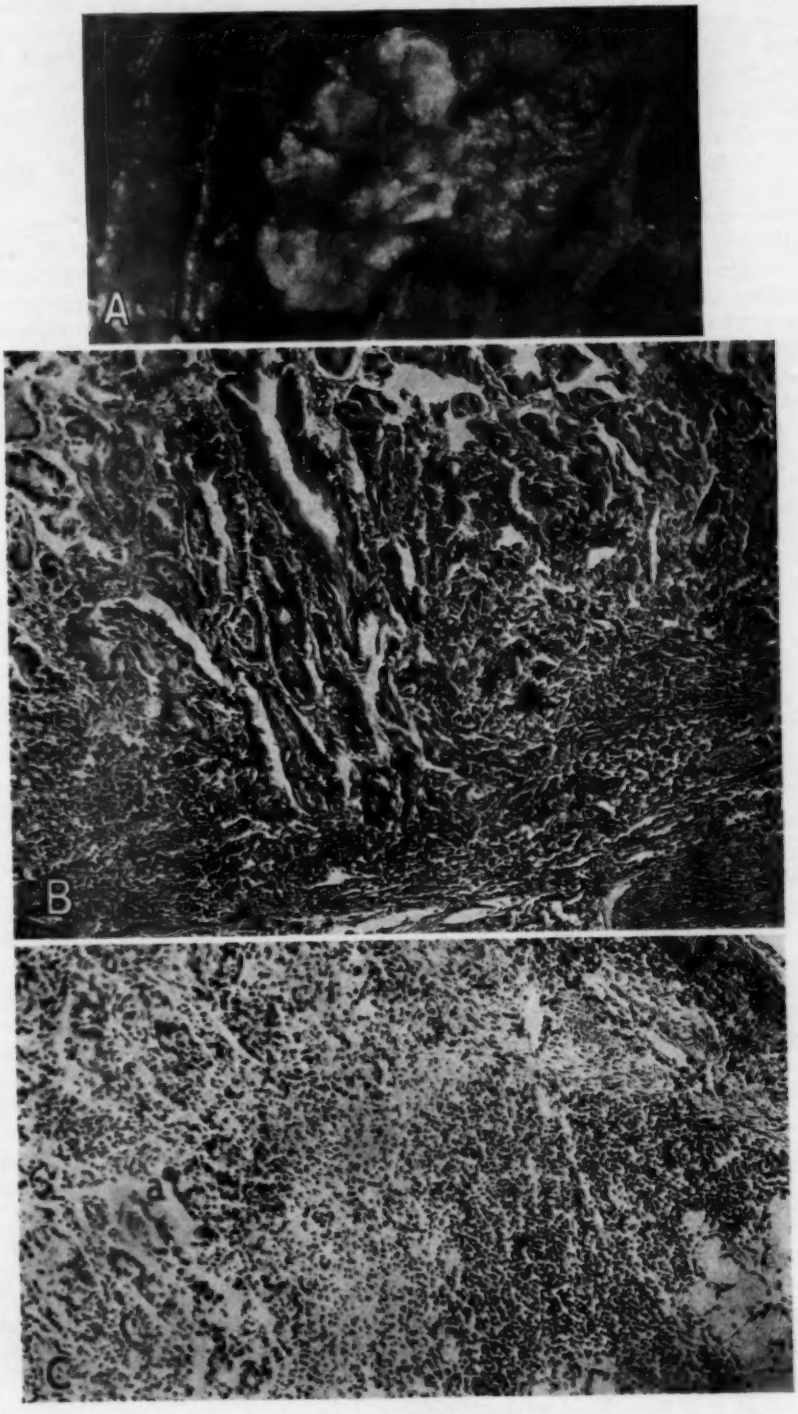
Histologic study of the duodenal ulcers revealed no evidence of cancer. Typical mucosal erosion was present, the epithelium and the glands at the margins being quite normal in appearance. At the margin of the tumor there was marked proliferation of the epithelium, with bizarre and atypical gland formation. The lining cells were large and actively secreting, and the nuclei were markedly hyperchromatic. The appearance was that of a well differentiated adenocarcinoma which projected for the most part on the surface but which also invaded the wall as far as and including the superficial portion of the inner circular muscularis. The more centrally located part of the tumor was ulcerated; it infiltrated the intestinal wall as deep as the serosa and was distinctly anaplastic. Most of the neoplastic cells were scattered as individual cells or as small niduses, with occasional abortive attempts at formation of glands. Groups of Brunner's glands, perfectly normal, were seen in or about the margins of the tumor. A moderate amount of round cell infiltration was present in the deeper portions of the intestinal wall. There was no evidence of regional or distal metastases.

Sections of the adenomatous polyps of the colon revealed considerable irregularity in size and shape of the glands, with cystic dilatation of some. All of the glands were of the active mucus-secreting type, and many of the superficial ones displayed marked nuclear hyperchromatism. The submucosal lipoma was well localized and covered by intact mucosa.

EXPLANATION OF FIGURE

A, primary infrapapillary adenocarcinoma of the duodenum. *B*, section taken from an edge of the tumor shown in *A*. The neoplastic glands extend into the superficial portions of the inner circular muscularis. *C*, section from the central ulcerated portion of the intestinal tumor. At this point the entire wall was infiltrated by tumor cells as far as the serosa. Note the more anaplastic type of growth (*a*), although there is still some attempt at gland formation. Brunner's glands are seen at the lower right hand corner of the picture and the intervening tissue is infiltrated by lymphocytes.

From the Department of Laboratories and Research, Bronx Hospital.



(See legend on opposite page)

The renal tubular epithelium was the seat of cloudy swelling, and the endothelium of the glomerular tufts was swollen so that in many instances the capillaries were bloodless. Some glomeruli were completely hyalinized. The larger renal vessels were moderately sclerosed.

In the lungs there was some atelectasis with edema and gray hepatization.

The rarity of infrapapillary carcinoma of the duodenum is shown in the accompanying table, only 47 instances having been reported, including the present case. Lieber, Stewart and Lund [1] stated that the incidence at necropsies is 0.01 to 0.1 per cent. Eger [2] reported an incidence at necropsies for all types of duodenal carcinoma of 0.036 per cent in 2,780 cases, and Hoffman and Pack,[3] an incidence of 0.033 per cent in 350,286 autopsies. Allen [4] noted 11 instances of carcinoma of the duodenum among 154,613 admissions, a ratio of 1:14,000. In a study of 228 cases of carcinoma of the small intestine, Hoffman and Pack found the relative frequency of duodenal involvement to be 45.6 per cent (104 cases). Browne and McHardy [5] in an analysis of 134 cases of neoplasm of the small bowel found that the duodenum was the site of involvement in 51 (37.3 per cent), the ileum in 44 and the jejunum in 39. Dardinski [6] noted 147 cases of primary carcinoma of the small intestine in a total of 6,882 cases of carcinoma of the bowel.

The associated lesions in the present case are illustrative of similar observations made by us over a period of years. It is almost axiomatic that intestinal

Reported Cases of Infrapapillary Carcinoma of the Duodenum

	Author	Cases
1.	Hoffman and Pack [3]	6
2.	Lieber, Stewart and Lund [1] *	30
3.	Howes, W. E.: New York State J. Med. **37**:1907, 1937	1
4.	Allen [4]	2
5.	Claiborn, L. N., and Dobbs, W. G. H.: Surgery **4**:97, 1938	1
6.	Pollack, S.: Radiology **31**:362, 1938	1
7.	Handfield-Jones, R. M.: Lancet **2**:1168, 1938	1
8.	Bergendal, A.: Acta radiol. **20**:417, 1939	2
9.	Macindoe, P. H.: M. J. Australia **1**:48, 1940	1
10.	Cabot Case 27092: New England J. Med. **224**:386, 1941	1
11.	Felsen, J., and Wolarsky, W.: Arch. Path. **36**:428, 1943	1
		47

* Lieber, Stewart and Lund include 28 authentic cases collected from the literature and 2 of their own. The 6 cases of Hoffman and Pack were not included.

lesions are frequently multiple. Serious consideration must be accorded the possibility that in this case there may be some relationship between the multiple adenomas of the colon and the neoplasm of the duodenum. The most frequent simple tumor of the duodenum is adenoma. The tendency of the adenomatous polyp of the colon to become cancerous has long been recognized. According to some, carcinoma of the colon is superimposed on preexisting adenoma. This does not imply, however, that in every instance adenoma becomes cancerous. On the contrary, in most of the cases it remains silent and noncancerous (6.4 per cent in our series of 955 necropsies, including those on babies). In the case reported there appears to be a certain parallelism in the gross and histologic appearance between the duodenal tumor and the cancerous adenoma of the colon. Simple adenoma of the colon is a freely movable tumor which begins as a localized proliferation of the mucosal epithelium so that in the early stages the gross appearance is a small, flat, sessile, button-like elevation of the mucosa. As the tumor grows it takes on a rather characteristic delicate lobulation and acquires a pedicle of normal mucosa, probably due to attempts by the intestine at expulsion of the tumor as if it were

1. Lieber, M. M.; Stewart, H. L., and Lund, H.: Arch. Surg. **35**:268, 1937.
2. Eger, S. A.: Arch. Surg. **27**:1087, 1933.
3. Hoffman, W. J., and Pack, G. T.: Arch. Surg. **35**:11, 1937.
4. Allen, C. I.: Am. J. Surg. **40**:89, 1938.
5. Browne, D. C., and McHardy, G.: J. A. M. A. **115**:2257 (Dec. 28) 1940.
6. Dardinski, V. J.: Am. J. Path. **10**:313, 1934.

a foreign body. When cancerous changes supervene, the reverse occurs: The polyp gradually loses its pedicle, becomes more sessile and fixed to the mucosa, and undergoes central necrosis. The typical cancerous adenoma of the colon is a well defined, relatively superficial tumor with depressed center and rolled or everted margin. Microscopically, this tumor is well differentiated, grows slowly and remains fairly well localized for a long time. The general character of the duodenal tumor reported here bears a close resemblance to adenoma malignum of the colon. This impression is strengthened by the coexistence of adenomatous polyps of the colon, at least one of which exhibited suggestive early changes bordering on cancer. There is a great paucity of carefully studied intestinal material in the literature relative to the presence of multiple lesions.

Two types of duodenal carcinoma have been described—the localized, bulky, soft, ulcerated form and the circumscribing scirrhous type. The first is the more frequent and was encountered in the present case. The locations are best defined as being suprapapillary, peripapillary (most frequent type) and infrapapillary relative to the papilla of Vater. The infrapapillary type may occur in the descending, horizontal or ascending limb of the duodenal loop, occurrence in the last named being extremely rare and applicable to the present case.

No clinical history, symptoms or signs were elicited in this case to indicate the presence of duodenal disease. Like silent carcinoma of the colon, the lesions were revealed only incidental to a routine necropsy of a patient who obviously succumbed to an acute cerebral accident. Lieber, Stewart and Lund in their report of 30 cases of infrapapillary carcinoma of the duodenum noted that men were affected in 20 instances. The extremes of age were 35 and 74 years. The tumor was found to be 3 to 5 cm. in length in over half the cases. Metastases to adjacent structures were present in 50 per cent. All were diagnosed as adenocarcinoma. The chief clinical symptoms and signs were pain, vomiting and constipation alternating with diarrhea. Anorexia, gaseous eructation, nausea and loss of weight were associated manifestations. The location and the type of growth determine the clinical symptoms and signs, the peripapillary form tending to give rise to earlier symptoms, due to biliary stasis. Similarly, the circumscribing stenosing type results in obstructive intestinal symptoms. Pain is most often noted in the epigastrium or the right hypochondrium. Roentgenographic diagnosis is possible in a fair percentage of cases if a careful roentgenologic technic is followed in order to demonstrate defects in mucosal pattern. Unfortunately, the rarity of carcinoma of the infrapapillary portion of the duodenum does not encourage routine painstaking study of this portion of the bowel. Theoretically, intubation of the duodenum, using small amounts of barium sulfate followed by air insufflation, should prove helpful in suspected cases.

Therapy is entirely surgical. The operative mortality following resection is high. The duration of life in reported cases of infrapapillary carcinoma of the duodenum varies from five to nineteen months.

SUMMARY

A case of infrapapillary adenocarcinoma of the duodenum is described. The interesting features are its rarity, being the forty-seventh reported case, the complete absence of clinical symptoms and signs and the presence of multiple, and possibly related, intestinal lesions.

Laboratory Methods and Technical Notes

SUPERSATURATED SOLUTIONS OF FAT STAINS IN DILUTE ISOPROPANOL FOR DEMONSTRATION OF ACUTE FATTY DEGENERATIONS NOT SHOWN BY HERXHEIMER TECHNIC

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AND
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BETHESDA, MD.

The inadequacy of the widely used Herxheimer [1] acetone and 70 per cent alcohol solution of sudan IV for the demonstration of fatty substances has recently been brought forcibly to our attention. We had long recognized that this stain often materially reduced the amount of material glowing under crossed Nicol prisms but had not realized that it might destroy all trace of an acute fatty degeneration.

Following recent studies on oil-soluble dyes,[2] one of us (R. D. L.) had been using as a routine fat stain a solution of sudan brown (Colour Index No. 81, synthesized sample) in 30 per cent methanol (methyl alcohol), 30 per cent acetone and 40 per cent water. This gives deep brownish red staining of fats with considerable yellowish brown tinging of cytoplasm. The dilute acetone-methanol solution deteriorates rather rapidly and must frequently be prepared afresh from a stock solution in acetone and methanol. Precipitation is not especially troublesome but does occur at times. Not liking the brownish tone of this stain, the other of us (L. L. A.) continued to use the Herxheimer acetone and 70 per cent alcohol solution of sudan IV.

In an effort to preserve the doubly refractile material lost with the Herxheimer method and to remedy the defects of the sudan brown stain just described, we recently tried isopropanol (isopropyl alcohol) as a solvent for sudan IV.

A saturated solution of sudan IV (NZ-11) in 99 per cent isopropanol was made up, and from this solutions in 50, 60 and 70 per cent isopropanol were prepared by dilution with appropriate amounts of distilled water. Frozen sections of human adrenal cortex, of a cutaneous xanthoma and of cat kidney (the last normally contains quite numerous fat droplets in the epithelium of the cortical convoluted tubules) were stained for varying intervals in these several dilutions. The absolute isopropanol solution of sudan IV removed all fats in five minutes. The 70 and 60 per cent isopropanol solutions gave moderately deep orange fat, while the 50 per cent isopropanol solution fat was a distinctly paler and more yellowish orange. In ten minutes the color was distinctly deeper, and in one to two hours all three dilutions gave quite deep orange red fat. But, with the 60 per cent isopropanol possibly, and definitely with the 70 per cent there was a loss of both stainable fat and doubly refractile material in the one to two hour staining.

Consequently, the use of 70 per cent isopropanol was discarded and a ten minute staining in the 60 per cent isopropanol solution of sudan IV was adopted for comparison with a five minute staining in the Herxheimer acetone and 70 per cent alcohol solution. Some 40 different specimens containing fat were studied. Precipitates were frequent and troublesome with the Herxheimer solution but were generally absent with the 60 per cent isopropanol solutions. In every case in which fat was demonstrated with the acetone and 70 per cent alcohol solution, it was shown in approximately equal or definitely greater quantity with the 60 per

From the Division of Pathology, National Institute of Health.

1. Herxheimer, G.: Centralbl. f. allg. Path. u. path. Anat. **14**:891, 1903.

2. Lillie, R. D.: Study of Certain Oil Soluble Dyes for Use as Fat Stains, J. Tech. Methods, 1943, to be published.

cent isopropanol solution. Further, in a number of the cat kidneys the acetone and 70 per cent alcohol solution showed only clear vacuoles and some precipitate, but no fat, while with the 60 per cent isopropanol much fat was shown. Several rat kidneys showed moderate numbers of fine fat droplets in the epithelium of the proximal convoluted tubules with the 60 per cent isopropanol stain and none with the acetone and 70 per cent alcohol; others showed fat with both stains. Sixteen cat, chicken, rat and mouse livers showed numerous fine fat droplets in the liver cells with both stains, while 8 (including 1 human) showed them with the 60 per cent isopropanol stain and not with the acetone and 70 per cent alcohol, and in these livers the cells were large and granular in paraffin sections, without easily discernible vacuolation.

As successive batches of sections were stained during several days with the 60 per cent isopropanol solution the fat droplets became paler and paler orange. When the causes of this were sought for, it was found that the hitherto unused stock solution in the 60 per cent isopropanol solution had become definitely pale in color and that a considerable amount of residue had accumulated in it. Similar deterioration of solutions of sudan III in absolute alcohol after dilution to 70 per cent was reported by Kay and Whitehead.[3] When fresh dilutions to 50 and 60 per cent were made from the saturated solution in absolute isopropanol, the original brilliant orange red staining of fats was again attained. Saturated solutions prepared by adding an excess of dry sudan IV to previously mixed 60 and 70 per cent isopropanol dilutions and shaking at intervals for several days gave unsatisfactory pale orange stains or none whatever.

Hence it seemed indicated that a supersaturated solution was made by the technic first employed, and that such supersaturated solutions are required for prompt staining from isopropanol as dilute as 50 to 60 per cent.

To confirm and extend these findings, a large number of sections were cut from the kidney of one cat in which the fat was found to be entirely removed in five minutes by the acetone and 70 per cent alcohol stain and well shown by the 60 per cent isopropanol solution. Saturated solutions of sudan IV (CZ-5) in absolute alcohol, 100 per cent propanol, 99 per cent isopropanol and normal butanol (butyl alcohol) were prepared, also saturated solutions in 99 per cent isopropanol of sudan IV (NZ-11), of oil red 4B (Commonwealth Color and Chemical Company, no. 26870)[1] and of sudan brown. Dilutions of each to 50, 60 70 and 80 per cent were made by adding appropriate amounts of water to the saturated alcoholic solutions. The 50 per cent dilutions gave a heavy precipitate in all after five to ten minutes, the 60 and 70 per cent less, and the 80 per cent only a moderate precipitate. Sections were stained in the supernatant fluids for five and ten minutes and for one and two hours.

Consistently with the experience of Kay and Whitehead[4] with supersaturated 69 per cent alcohol solutions, a considerable amount of precipitate was formed in the sections stained with the dilute ethanols, but excellent deep orange fat staining was produced with 50 and 60 per cent alcohol in all time intervals, paler with the 50 per cent at five minutes. With longer than five minute staining the 70 per cent alcohol occasioned increasing loss of fat, leaving numerous clear vacuoles after the one and two hour staining periods. This solvent action was more pronounced with the 80 per cent alcohol, so that nearly all the fat was lost in one hour and none was demonstrable after two hours.

Propanol solutions gave fairly good orange red staining from the 50 and 60 per cent dilutions, with little precipitation before the two hour period. With 70 per cent dilution much of the fat was lost even in five and ten minutes, and with 80 per cent only a little was demonstrable after five minutes' staining and none with longer intervals.

Isopropanol solutions again gave brilliant staining results, a little deeper red wtih one of the sudan IV samples than with the other, a brilliant deep red with oil red 4B and a quite deep orange brown with sudan brown. In this series of dilutions the 50 per cent isopropanol gave brilliant results, and it was thought that the 60 per cent occasioned some loss of fat in two hours, while the 70 per cent definitely did, and usually little or no fat was demonstrable in any time interval after the use of 80 per cent isopropanol except in one of the five minute sections.

No fat was demonstrable with 50, 60, 70 or 80 per cent butanol solutions in any time interval.

COMMENT

While it has been generally recognized that stronger than 70 per cent alcohol as a solvent for sudan III or sudan IV was apt to occasion loss of fat, Romeis[5]

3. Kay, W. W., and Whitehead, R.: J. Path. & Bact. **39**:449, 1934.

4. Kay, W. W., and Whitehead, R.: J. Path. & Bact. **41**:303, 1935.

5. Romeis, B.: (*a*) Virchows Arch. f. path. Anat. **264**:301, 1927; (*b*) Ztschr. f. mikr.-anat. Forsch. **16**:525, 1929; cited by Romeis[5c] and Kay and Whitehead[4]; (*c*) Taschenbuch der mikroskopischen Technik, ed. 13, Munich, R. Oldenbourg, 1932.

has been almost the only advocate of the use of weaker alcohol solutions on account of fat losses. He pointed out that the usual saturated solutions in 70 per cent alcohol stain more vigorously if one adds 10 per cent water just before staining. This gives a distinctly turbid solution in about 64 per cent alcohol, which is supersaturated. He expressed a preference for the preparation of a saturated solution in 40 per cent alcohol by diluting with water a saturated solution in stronger alcohol and filtering twenty-four hours after dilution. Sections are stained in this six to twenty-four hours at room temperature or three to sixteen hours at 37 C. This procedure, he said, preserves even the finest lipoid droplets, but he did not note cases in which all of the fat present may be of this relatively easily soluble type.

In regard to the acetone and 70 per cent alcohol solution of Herxheimer,[1] Romeis [5c] warned against staining for much longer than two to three minutes, while Langeron [6] recommended fifteen to twenty minutes, Schmorl [7] twenty to thirty minutes, Mallory [8] two to five minutes and Conn [9] the same. Our previous experience has been that five to ten minutes gave adequate staining but that the acetone and 70 per cent alcohol solvent often definitely reduced the amount of demonstrable doubly refractile material as compared with that in sections stained with aqueous nile blue sulfate.

In regard to the widely used 70 per cent alcohol solutions of sudan III and sudan IV, Kaufmann and Lehmann [10] found that in sections of *Holundermark* (elder pith) impregnated with various purified fatty substances, more of these were demonstrable when 50 per cent alcohol was used as the solvent for sudan than when the dye was in 70 per cent alcohol and later [11] agreed with Romeis [5a] that his 40 per cent alcohol method was the best for showing maximal amounts of fat, as they found that 40 per cent alcohol extracted only 1.5 per cent of the fatty acids from a fatty liver in twenty-four hours whereas 70 per cent alcohol removed 25.5 per cent in twenty minutes.

Escher [12] first recommended a solution of sudan IV in 50 per cent propanol and Gross [13] found that excellent staining was obtained with solutions in 50 per cent propanol or 50 per cent technical isopropanol. Gross preferred a saturated solution of sudan IV in previously mixed 50 per cent diacetin (1,3-glyceryl diacetate) in water. This dissolves more sudan IV, is practically not volatile and is not a fat solvent. He noted that addition of small amounts of acid caused gradual hydrolysis of the diacetin, which impaired the stain. In our hands this method at first gave excellent results, but on routine use the staining gradually deteriorated, so that after some weeks we abandoned the method. Doubly refractile lipoids were preserved by this method. After we discontinued the use of the solution, it was noted that it had developed a definite odor of acetic acid.

Proescher's [14] oil red O (sudan II) solution in 70 per cent pyridine, which is said not to dissolve fats or to produce precipitates, stains promptly and intensely. Its odor is highly objectionable for routine use. We have not explored its solvent action on the more easily soluble fats.

In conclusion, the following basic technic may be recommended.

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6. Langeron, M.: Précis de microscopie, Paris, Masson & Cie, 1934.
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TECHNIC

A stock saturated solution of sudan IV, oil red 4B or sudan brown is made in isopropanol (99 per cent isopropyl alcohol). For a day's staining, take 3 cc. of stock solution and mix with 3 cc. or 2 cc. of distilled water (for 50 or 60 per cent isopropanol, respectively), let stand five minutes, and filter. The 60 per cent dilution is unusually good all day; the 50 per cent may get too weak after three or four hours. Stain frozen sections ten minutes in this solution (five minutes will suffice for 60 per cent isopropanol), float out in tap water, stain two to five minutes in alum-hematoxylin containing 2 per cent acetic acid (Mayer's acid hemalum will serve, or a 1:3 or 1:4 dilution of Lillie's modification in 2 per cent acetic acid), rinse in water, blue in 1 per cent disodium phosphate, mount in Apathy's gum syrup.[15]

CONCLUSIONS

The preparation and use of supersaturated solutions of sudan IV, oil red 4B and sudan brown in 50 and 60 per cent isopropanol as described demonstrate fatty substances which may be partly or even entirely lost when 70 per cent ethyl alcohol and acetone are employed as the solvent, or even 70 per cent alcohol alone. Precipitation is also less with the isopropanol solutions. By this method sudan IV gives to fats a deep orange red color, oil red 4B a purer red, sudan brown a deep orange brown.

15. Dissolve 50 Gm. of acacia (gum arabic) and 50 Gm. of cane sugar in 100 cc. of distilled water at 55 to 60 C. with frequent shaking. Restore the volume with distilled water. Add 1.5 cc. of 1 per cent merthiolate as a preservative (or 100 mg. of thymol). Place in a vacuum chamber for a few minutes to remove air bubbles. This formula is a modification of that described by Mallory.[8]

Books Received

THE PRINCIPLES AND PRACTICE OF INDUSTRIAL MEDICINE. Edited by Fred J. Wampler, M.D., professor of preventive and industrial medicine, Medical College of Virginia. Pp. 579, illustrated. Price $6. Baltimore: The Williams & Wilkins Company, 1943.

This book is the joint product of 33 contributors selected because of their interest and competence in different branches of industrial medicine. Medical students and physicians have here an excellent guide in the study of principles and methods of modern medical care of workers in diverse industries. Louis Schwartz in his chapter on occupational diseases of the skin summarizes well the main facts of occupational or industrial cancers, especially those of the skin. The knowledge of occupational cancers is constantly increasing. They are of special interest to investigators because of the light they throw on the mechanisms of human cancer. They are of particular concern from the practical point of view because so largely preventable by the elimination of carcinogenic agents from industrial operations. For these reasons, in the preparation for the next edition of the book, consideration should be given to a separate chapter on the relation of occupation to cancer and to the means of its prevention.

THE EPIDEMIOLOGY OF DIPHTHERIA DURING THE LAST FORTY YEARS. W. T. Russell. Medical Research Council, Special Report Series no. 247. Pp. 52. Price 1 shilling. London: His Majesty's Stationery Office, 1943.

An important statistical review and study of the morbidity, the mortality and the types of diphtheria and of active immunization against the disease as practiced in Canada, the United States and England. The evidence is "impressively in favour of active immunisation against diphtheria as a life-saving measure. . . ."

COLLECTED PAPERS OF THE MAYO CLINIC AND THE MAYO FOUNDATION. Edited by Richard M. Hewitt, M.D.; A. B. Nevling, M.D.; John R. Miner, Sc.D.; James R. Eckman, A.B., and M. Katharine Smith, B.A. Volume XXXIV, 1942, published in July 1943. Pp. 999, illustrated. Price $11. Philadelphia and London: W. B. Saunders Company, 1943.

THE PHARMACOLOGY OF THE OPIUM ALKALOIDS. PARTS 1 AND 2. Hugo Krueger, assistant professor of pharmacology, St. Louis University School of Medicine, formerly research assistant professor of pharmacology, University of Michigan; Nathan B. Eddy, principal pharmacologist, United States Public Health Service, formerly research professor of pharmacology, University of Michigan, and Margaret Sumwalt, associate physiologist, United States Public Health Service, formerly research instructor of pharmacology, University of Michigan. Pp. 1440. Price $3. Supplement no. 165 to the Public Health Reports. Washington, D. C.: United States Government Printing Office, 1943.

TEN YEAR REPORT, OCT. 1, 1932 TO SEPT. 30, 1942, OF THE INSTITUTE FOR PSYCHOANALYSIS, DEDICATED TO INCREASING THE KNOWLEDGE OF THE PSYCHIC PROCESSES OF MAN. Pp. 80. Chicago: Institute for Psychoanalysis, 1943.